Oxymax for Windows

User Manual

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Chapter 1 - Introduction

1.1 Overview

Columbus Instruments' Comprehensive Lab Animal Monitoring System (CLAMS) brings together a diverse range of physiological and behavioral instrumentation into a simply controlled system capable of monitoring up to 32 lab animals. CLAMS is based on Columbus Instruments' original Oxymax open-circuit calorimeter. Oxymax serves as the central platform onto which clients may add activity, feed monitoring, feed control, telemetry and more. These elements are tied together using the CI-Bus communication system that allows easy integration of all CLAMS components.

This manual contains instructions for the operation of the Oxymax for Windows software. The CLAMS Hardware manual contains the specifications and function of the hardware components in the CLAMS family. Since many CLAMS installations are considered custom, not all sections of either manual will be relevant to every customer. This manual, when viewed completely, covers material applicable to nearly every CLAMS user.

> **Note**
> Screenshots and diagrams presented in this manual may be resized or edited to fit the format of the page. Do not expect all figures to be verbatim representations of the actual Oxymax for Windows software.

1.2 System Requirements

The minimum system requirements for a computer to run Oxymax for Windows are:

- Windows-based PC running Windows XP/Vista/7/8
- 512MB RAM (1GB Recommended)
- Hard drive with at least 100MB of free-space for installation
  - Space for acquired data may vary; at least 1GB recommended
- CD-ROM drive (for installation)
- Monitor running minimum resolution of 1024 x 768
- At least one free RS-232 Communications Port or a registered USB-to-Serial Adapter
  - Most new systems include the USB-to-Serial Adapter used to test and setup the system at Columbus Instruments

1.3 Preparing for Installation

To ensure that Oxymax for Windows performs reliably there are a small number of Windows settings that should be configured before installation.

- **Windows XP** – Depending on the manufacturer of the PC, sometimes power-saving settings are in place to put Windows to sleep after long periods of inactivity. Sleeping will interrupt and halt any currently running Oxymax experiments and should be disabled on computers running Oxymax experiments.

Note – Windows XP End of Life

Microsoft has ended support for Windows XP as of April of 2014. Some newer releases of Oxymax for Windows may cease to function under Windows XP and it's recommended that users upgrade to at least Windows 7.

- **Power Options** – The power settings for Windows XP can be found in "Control Panel >> Power Options". The option for the Control Panel can usually be found in the Start Menu.
  - **Desktop Users** – If the PC is a desktop, the action that is required is setting the option for "Turn off hard disks" to "Never".
  - **Laptop Users** – Laptop users should always perform experiments with the laptop lid open and plugged into an AC outlet. The options "Turn off hard disks", "System Standby" and "System Hibernate" should all be set to "Never" under the "Plugged In" column.

- **Windows Vista/7/8 Users** – All of the power settings that would need to be changed under Windows XP also need changed for Vista/7 but are found in different places. Additionally, Oxymax needs to be able to run by a user with *administrative privileges without restriction* in order to properly save data.
  - **Power Options** – The power settings for Windows Vista/7 can be found in "Control Panel >> Power Options". The option for the Control Panel can usually be found in the Start Menu. When the Power Options screen has loaded, select "Change Plan Settings" next to the currently selected power plan. It's recommended that the first plan ("Recommended Settings") be used.
    - **Desktop Users** - Set the "Put the computer to sleep" option to "Never".
    - **Laptop Users** – Laptop users should always perform experiments with the laptop lid open and plugged into an AC outlet. Set the option "Put the computer to sleep" to "Never".
  - **User Account Control (UAC)** – Starting in Windows Vista, Microsoft implemented a number of security features in a suite called "User Account Control" designed to prevent programs from making changes to sensitive files without the user's knowledge. These security features also inadvertently disrupt intended behavior in '"legacy" software like Oxymax for Windows. To ensure that Oxymax for Windows behaves as expected, UAC should be disabled for computers that communicate with CLAMS hardware.
    - **Windows Vista** – Go to "Control Panel >> User Accounts" and select "Turn User Account Control on or off". Click "Continue" if prompted by Windows. Uncheck the box that says "Use User Account Control (UAC) to help protect your computer" and click "OK".
    - **Windows 7/8** – Go to "Control Panel >> User Accounts" and select "Change User Account Control settings". Click "Continue" if prompted by Windows. Move the vertical slider down to "Never notify" and click "OK".

1.4 Installation

The Oxymax for Windows installation disc contains configuration information that is specific to the system or systems for which it was created. If the disc is misplaced, Columbus Instruments keeps all customer Hardware Configurations on file; contact Columbus Instruments for a new installation disc.

If installing from a CD, the installation disc may or may not automatically load the Oxymax for Windows installer upon insertion depending on the computer's settings. If it does not, click "Start >> Run" and select `E:\setup.exe` (where `E:\` is the letter of the computer's CD-ROM drive).

If installing from a downloaded package, unzip the contents and then run `setup.exe`.

When the installer finishes loading, it will pause on the window in Figure 1.

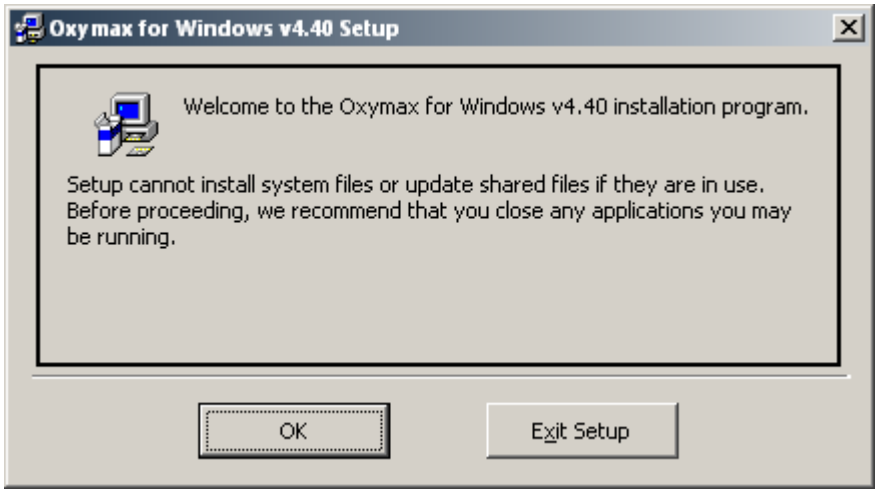

Oxymax for Windows v4.40 Setup
Welcome to the Oxymax for Windows v4.40 installation program.
Setup cannot install system files or update shared files if they are in use. Before proceeding, we recommend that you close any applications you may be running.
OK
Exit Setup

Figure 1 - Installation Welcome Screen

Selecting "OK" will proceed to the next step of the setup: picking an installation directory. The default directory is `C:\Program Files\Oxymax\`, but it can be changed if necessary. Clicking the large button in Figure 2 is the last step in installing Oxymax for Windows.

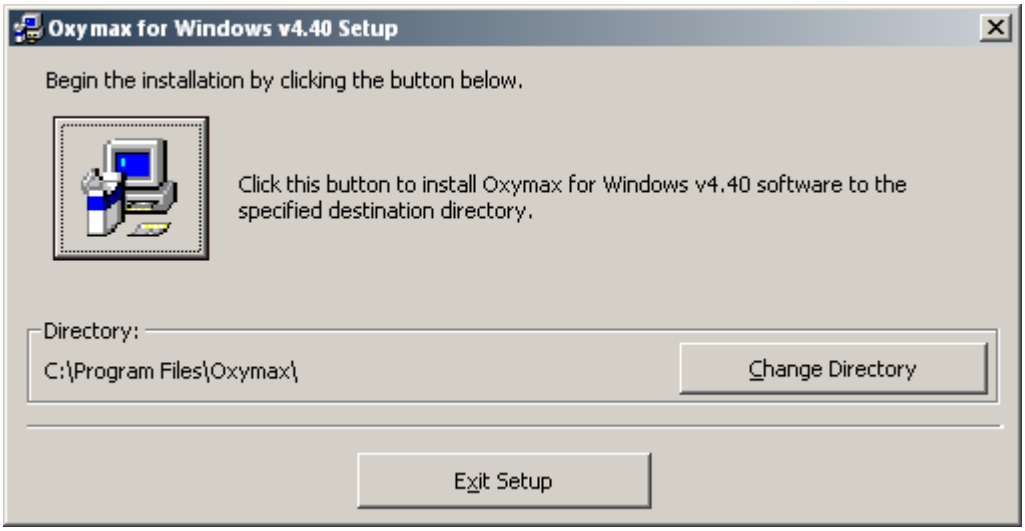

Oxymax for Windows v4.40 Setup
Begin the installation by clicking the button below.
Click this button to install Oxymax for Windows v4.40 software to the specified destination directory.
Directory:
C:\Program Files\Oxymax\
Change Directory
Exit Setup

Figure 2 - Directory Selection Window

Before the end of the installation, the installer may prompt to "Overwrite Configuration" if this is an update. This will copy the contents – instruction manuals, etc -- of the included "Config" folder to the Oxymax directory. Also, the installer may prompt to install "communication drivers" in the event that the system uses an included USB-to-Serial Adapter. Installing these drivers is recommended if it has not been done before.

When the installation finishes, shortcuts for the installed software are placed in the Start Menu under "Start" >> "Programs" >> "Columbus Instruments". Oxymax for Windows also comes with a troubleshooting program called "CI Diagnostic" that only needs to be used to troubleshoot issues with a Columbus Instruments Technician.

Chapter 2 - Starting Oxymax for Windows

2.1 Hardware Configuration

Oxymax for Windows is the software used to setup experiments and collect data from a CLAMS system. Each CLAMS system ships with an installation disc that contains Hardware Configuration information specific to that system. Assuming the program is installed in the default directory, the system's configuration is stored in `C:\Program Files\Oxymax\Config\` or `C:\Program Files (x86)\Oxymax\Config\` depending on the version of Windows in use (32 and 64-bit, respectively).

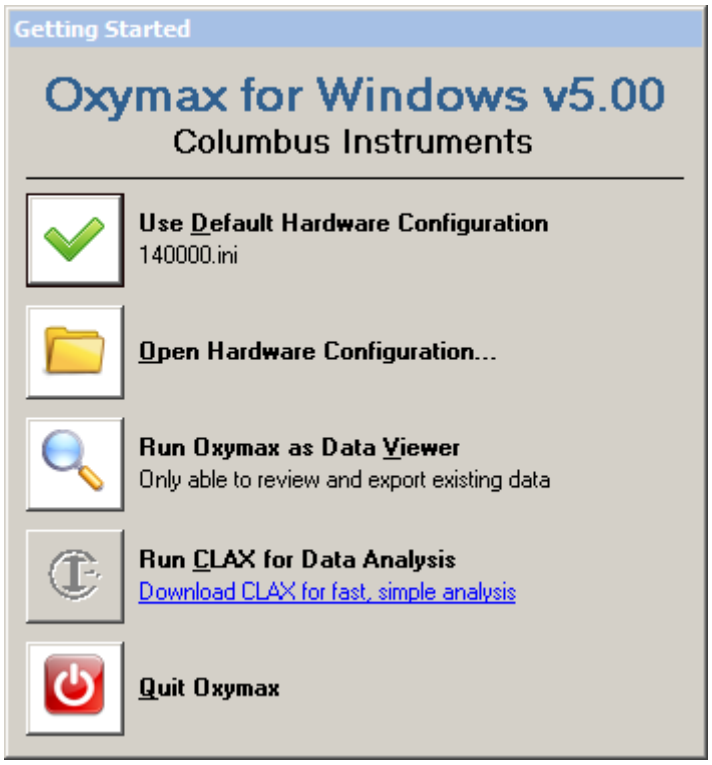


Figure 3 - Getting Started

When Oxymax launches, the Startup window shown in Figure 3 appears. There are five options for proceeding when Oxymax has been launched:

1. **Use Default Hardware Configuration** – Opens the displayed Hardware Configuration file without prompting.
2. **Open Hardware Configuration** – Brings open the selection dialog shown in Figure 4 to select a Hardware Configuration. Selecting "Yes" to the prompt reading "Make this the default hardware configuration" will replace the current default with the most recently selected file.
3. **Run Oxymax as Data Viewer** – Does not setup any of the hardware communication components of Oxymax and allows for the opening of existing data-sets only.

4. **Run CLAX for Data Analysis** – If installed, this will launch the CLAMS Examination Tool (CLAX) for data analysis. If not installed, there's an option for internet-connected PC's to download CLAX for free from the Columbus Instruments website.
5. **Quit Oxymax**

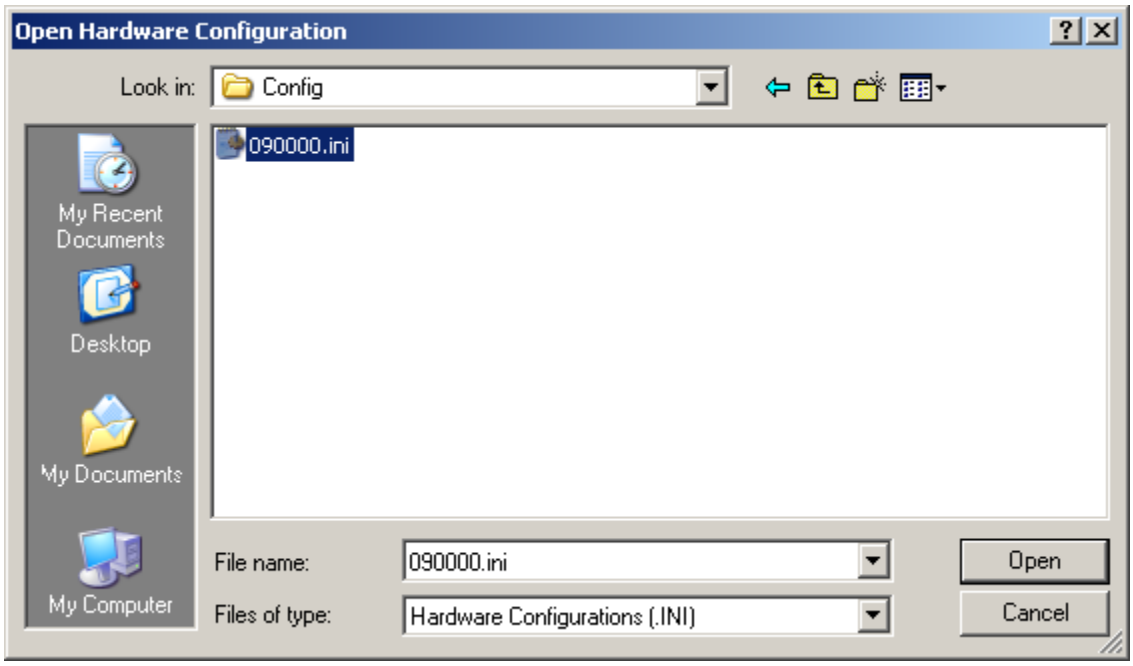


Figure 4 - Select Hardware Configuration

Note – Do not edit the Hardware Configuration file

Hardware Configuration files are written explicitly for the system as it is installed and configured. Should the system ever be changed or upgraded, a Columbus Instruments representative will alter and/or replace the Hardware Configuration file.

After selecting a Hardware Configuration, Oxymax will detect and initialize the Columbus Instruments hardware specified in that configuration and present a loading screen as in Figure 5. Oxymax for Windows will automatically detect the serial communications (COM) port the CLAMS system is connected to.

Figure 5 - Oxymax Initialization

2.2 Hardware Status

While Oxymax for Windows is searching for CLAMS hardware, it will display the "Oxymax Hardware Status" window that will remain suppressed unless the software fails to detect a required component. If an error is detected, the status window pictured in Figure 6 will appear.

The "Oxymax Hardware Status" window is divided into two tables that display a complete listing of the system's Oxymax and CLAMS hardware. The refresh buttons in each frame are used to attempt to communicate with the listed hardware. "Oxymax Hardware" contains a listing of all calorimetry-related hardware. "CLAMS Hardware" lists all hardware relating to feeding, drinking, activity and other features.

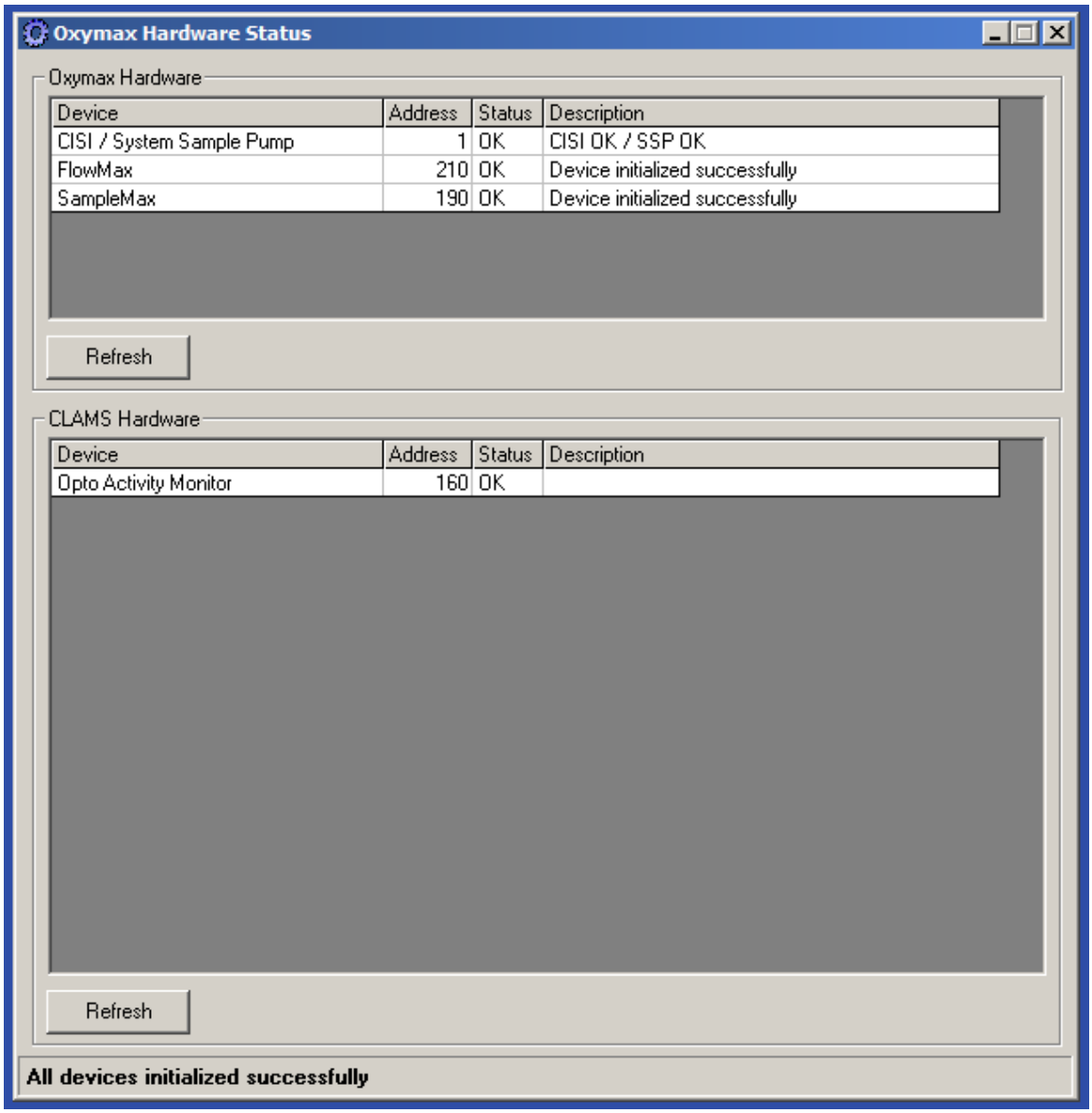


Figure 6 - Oxymax Hardware Status

In Figure 6, Oxymax for Windows attempted to connect to the hardware of a simple four-channel CLAMS system. Successfully detected hardware will be listed as "OK" whereas undetectable or uninitialized hardware will be listed as "Fail". The reason for the device failure is listed in the "Description" column of each table. Common error descriptions include:

- **No Response** – The device did not respond to communication at all. This is likely because it is powered off or disconnected from power, communication, or both.
- **Power Fail** – Usually pertaining to Balance devices, this indicates a failure to respond from the balance. This is usually the result of the balance being powered off; if the display is blank or reads "OFF", just tap the blue "Tare" button to turn the balance on. If this does not turn the unit on, check the power cable on the back to make sure it's secure.
- **Checksum Error, Response Error** – Some serial RS-232 adapters are not robust enough to provide consistent communication with a CLAMS system. Try updating the drivers for the unit in-use or replacing with a different model.

After the software is able to successfully detect all of the system's specified hardware, the "Oxymax Hardware Status" window can be minimized.

> **Hardware Statuses**
> Devices that are listed as "Fail" on the status window will **not** be polled during an experiment which will mean missing measurements and/or incorrect system operation. It's advised that all initialization errors be addressed and refreshed before proceeding to an experiment.

2.3 Main Window Navigation

After all of the hardware has been detected and initialized, the main Oxymax for Windows window will appear as it does in Figure 7. The "Oxymax Hardware Status" window can remain minimized in the corner of the application for the duration of the systems use unless there is a perceived problem with the system's communication.

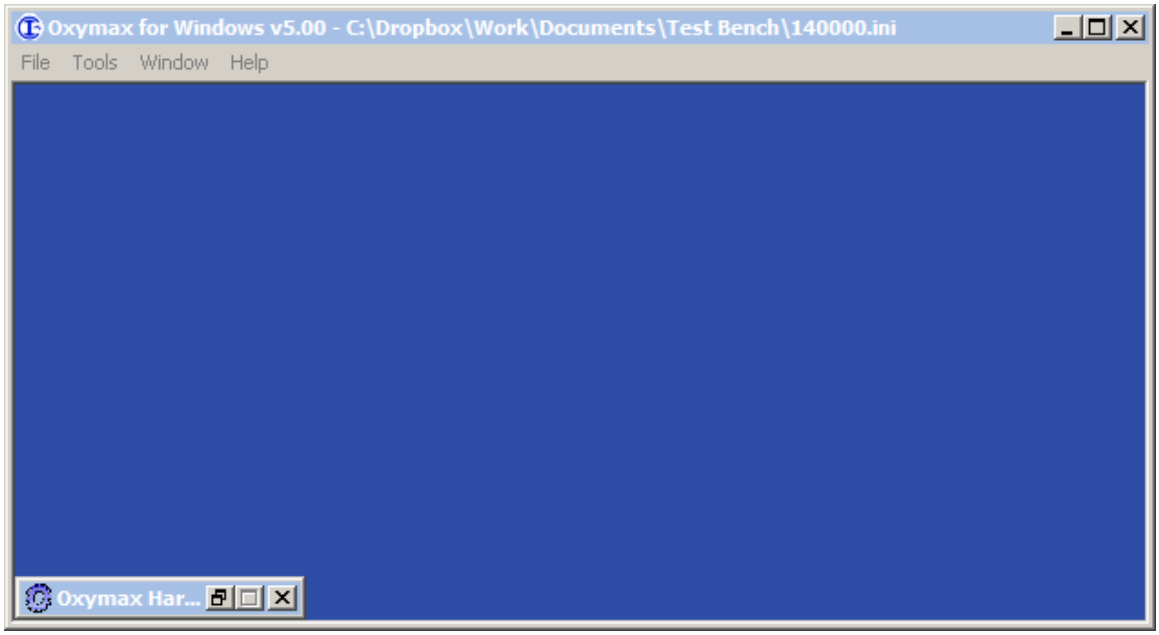


Figure 7 - Main Oxymax for Windows Window

The Main Window's workspace is a container for all Oxymax for Windows features and settings. These options are accessible from the menu pictured at the top of Figure 7. The menu options change depending on the feature that's in use. After a successful system initialization, the Main Window's menu options are:

- **File**
  - **Open Experiment Configuration** – Creates a blank data (CDTA) file from the selected Experiment Configuration.
  - **Open Experiment Data** – Opens a previously created CLAMS Data File. The acquired data can then be reviewed, graphed or exported.
  - **View Hardware Configuration** – Displays the currently loaded Hardware Configuration for review.
  - **Last Used .EXP** – If available, automatically loads the more recently used Experiment Configuration file.
- **Tools**
  - **Calorimetry** – All of the utilities relevant to the Oxymax calorimeter.
    - **Oxymax Utility** – Tool used to test the system's valves, sensors and flow controllers.
    - **Calibration** – Opens the Oxymax Calibration Utility for calibrating and testing the system's gas sensors.
  - **Feeding**
    - **Balance Utility** – Form used to refill feeder balances during an experiment.
    - **Load Cell Utility** – Form used to refill overhead load cell feeders during an experiment.
    - **Feed Control**

        - **Open Feeders** – Allows all animals' access to all feeders.
        - **Close Feeders** – Denies all animals' access to all feeders.
        - **Stop Feed Control** – Stops the set feeding algorithm for the running experiment.
    - **Activity Monitor** – Real-time display of activity beams for all chambers.
    - **Drinking**
        - **Purge** – Purges the intake line.
        - **Pump Status** – Displays the current status of the system's Volumetric Drinking Monitors.
    - **Urine**
        - **Balance Utility** – Form used to monitor urine balances during an experiment.
        - **Load Cell Utility** – Form used to monitor load cell urine monitors during an experiment.
        - **Urine Chillers**
            - **Chill** – Sets all Urine Chillers to chill their samples.
            - **Thaw** – Sets all Urine Chillers to thaw their samples.
            - **Off –** Turns off all detected Urine Chillers.
    - **Weight Utility** – Tool for testing animal weight fixtures.
    - **Iso-Thermex Temperature Utility** – Diagnostic utility for reading temperatures from all Iso-Thermex probes.
- **Window** – Contains a list of all currently open windows.
- **Help**
    - **Documents** – Lists PDF manuals and instructions for various procedures.
    - **Video Help** – Contains instructional videos.
    - **About** – Displays the Oxymax for Windows About Window.

Chapter 3 - Configuring an Experiment

3.1 Exploring Experiment Settings

To start or setup an experiment, Oxymax for Windows must have an Experiment Configuration file. CLAMS systems ship with a default configuration for each Hardware Configuration. The Experiment Configuration is named to match the Hardware Configuration with which it is meant to be used. The Experiment Configuration contains measurement interval lengths, units, subject information and other settings for an experiment.

To open an Experiment Configuration file, click "Open Experiment Configuration" from the menu and select the desired file. Oxymax creates a blank Experiment Window configured with the options specified in the selected Experiment Configuration as in Figure 8.

> **Note – Every CLAMS system is different**
>
> The blank data file you see with your configuration will most likely not look exactly like Figure 8. The example configuration may contain more or fewer measurement options that your system.

Figure 8 - Blank Data File

After opening an Experiment Configuration, the menu contains options for the configuration and control of the currently opened experiment. Not all of the options listed here need to be customized in order to properly run an experiment. Additionally, some options will only make themselves available if the CLAMS system supports them.

- **File**
  - **Save Experiment** – Saves any changes to the currently open Experiment Configuration.
  - **Save Experiment As** – Save the current Experiment Configuration under the chosen filename.

- **Export** – Contains options for exporting collected data. Full use of this feature is discussed in **5.2 Exporting Data**.
- **Close** – Closes the currently open Experiment Configuration.

- **Experiment**
  - **Setup** – Opens the Experiment Setup window for setting and configuring cage and data options. This feature is discussed further in **3.2** .
  - **Properties** – Opens the Experiment Properties window used in configuring measurement options for the specified CLAMS hardware. This feature is discussed further in **3.3 Measurement Configuration**.
  - **Calibrate** – Opens the dialog for calibrating the system's gas sensors. The calibration procedure is discussed in **4.2 Gas Sensor Calibration**.
  - **Run** – Starts the currently open experiment. This option is unavailable until a save location for experiment data has been selected.
  - **Stop** – Stops the currently running experiment.
  - **Add Event** – While an experiment is running, this option allows the user to make a note of something that occurred in the experiment for later review.
- **Tools**
  - **Feeding**
    - **Refill Feeder Balances** – Opens the utility for refilling balance-based animal feeders while an experiment is running.
    - **Refill Feeder Load Cells** – Opens the utility for refilling load-cell feeders while an experiment is running.
    - **Feed Control**
      - **Open Feeders** – In systems with feed access control equipment, this opens all of the feeders in the system.
      - **Close Feeders** – In systems with feed access control equipment, this closes all of the feeders in the system.
  - **Activity Monitor** – Real-time display of activity beams for all chambers.
  - **Treadmill Controllers**
    - **Start Treadmill Schedule** – In systems with treadmill controllers, this will start the user-specified speed schedule.
    - **Stop Treadmill Schedule** – In systems with treadmill controllers, this will stop the user-specified speed schedule and set the treadmill speed to the specified cool-down speed.
    - **Release Treadmill Control** – Enables manual control of the treadmill from the unit itself.
      - **Warning** – The treadmill will immediately change speeds to the value set by the knob on the front; make sure the knob is in a reasonable position or the treadmill is empty before returning control of the unit.
  - **Urine Chillers**
    - **Set Chillers to Chill** – Begins chilling samples in all Urine Chillers.
    - **Set Chillers to Thaw** – Begins thawing samples in all Urine Chillers.

    - **Turn Chillers Off** – Disables all detected Urine Chillers.
- **View**
    - **Hardware Configuration** – Opens the current Hardware Configuration file for review.
    - **Experiment Configuration** – Opens the current Experiment Configuration file for review.
- **Window** – Contains a list of all currently open windows.

The tabs on the Experiment Window separate the different kinds of acquired CLAMS data. Viewing and understanding acquired data is discussed later in **5.1 Understanding Data**.

3.2 Experiment Setup

Some of the settings contained in the Experiment Configuration, like measurement time or feeding bout thresholds, are persistent settings that remain the same for any instance of the same experiment. However, some settings, like subject identifiers and output file locations, need to change each time the experiment is run. These settings are presented on the Experiment Setup Window.

The Experiment Setup Window can be reached from the "Experiment >> Setup" menu after a blank data file has been created. An example of an Experiment Setup Window appears in Figure 9.

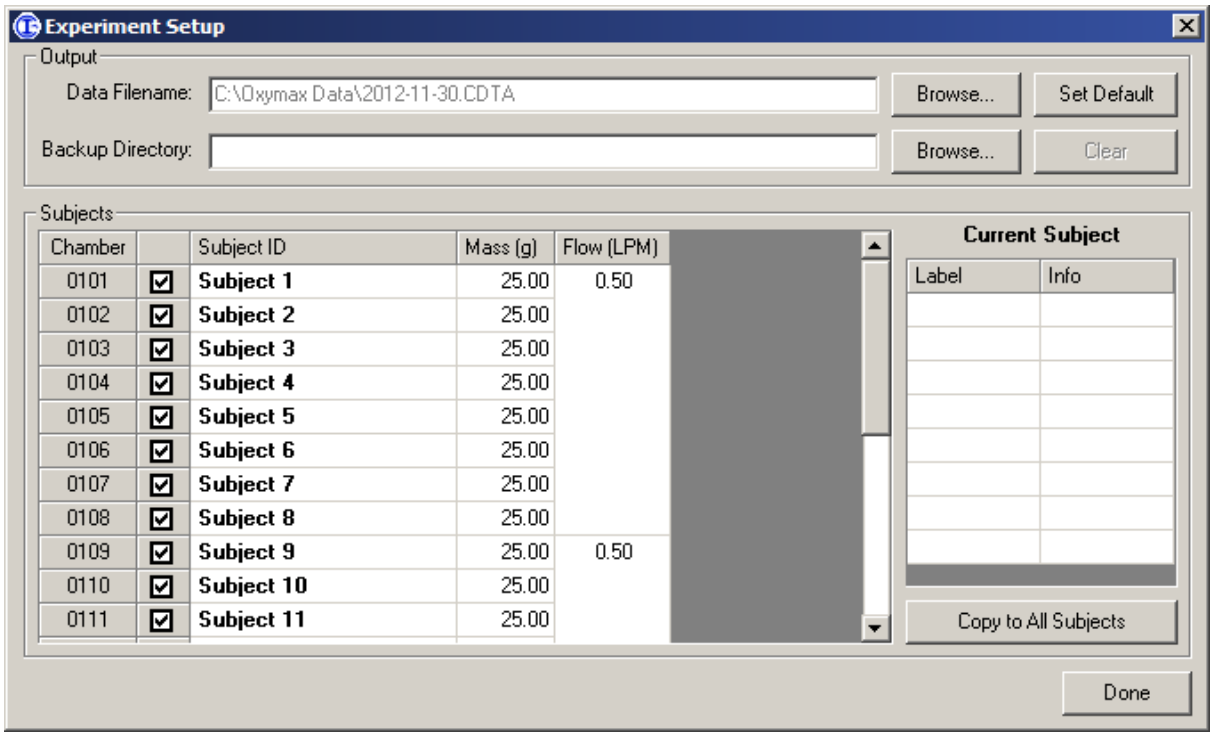


Figure 9 - Experiment Setup Window

Many of the settings required to start an experiment persist from experiment to experiment. The only setting that the user *must* set before starting an experiment is the

"Data Filename" setting. The Data Filename indicates the name and location that the data from the experiment will be saved. Clicking "Browse" opens a dialog window to select a save location for experiment data.

> **Note – Save Location**
> It is recommended that experiment data be saved outside the Oxymax directory and out of "Program Files" entirely. Windows considers "Program Files" a sensitive area and may not allow for access to that data after an experiment. Select another location (My Documents, Desktop, etc) on the local computer to save experiment data.
>
> A dedicated directory on the root drive – C:\ – is a good place to keep data where all users of the CLAMS system can store data without running into permissions issues.

If a "Backup Directory" is specified, Oxymax for Windows will make a duplicate of all experiment data and save it in that location. The "Clear" option resets the Backup Directory and disables data backup. It's recommended that a hard drive on a remote computer or server be used as a backup location in the event of a computer failure.

The roster of subjects to be included in the current experiment appears as a table on the left-hand side of the window. Each cage in the system appears as a row in this table with the following properties:

- **Chamber** – This numerical identifier is comprised of the group to which the chamber belongs as well as its position within that group. A "group" is defined as a collection of chambers that share gas sensors. Systems with multiple sets of gas sensors can take samples from multiple chambers at once, but each chamber is always sampled from only one set of sensors. In the example system presented in Figure 9, the system is comprised of eight chamber and one set of gas sensors (Oxygen and Carbon Dioxide).
- **Enabled** – Checking or unchecking this box will add or remove this chamber from service before the next experiment. This is useful when experiment groups are smaller than the CLAMS system or when it's necessary to shorten the time in between samples for each chamber. Subjects cannot be added or removed while an experiment is running.
- **Subject ID** – This is the animal-specific identifier associated with the subject that's going to be used in this chamber. It's a good idea to use unique Subject ID's for each subject, though Oxymax does not enforce this as a rule.
- **Mass** – This should be the subject's body mass taken immediately preceding an experiment. Metabolic rates are automatically normalized to body mass and this measurement is used in their calculation (more on that in **Appendix A – Oxymax Calculations**)
- **Flow** – This is the fresh air will be delivered to the chamber. Depending on the allocation of flow controllers in a system, some chambers share flow controllers and therefore will always have the same rates. In those cases, the cell for fresh air will

span the row of every chamber connected to that controller. Common flow rates are between *0.5 and 0.6 LPM (liters per minute) for mice and 2.0 to 3.5 LPM for rats*.

To edit any property within the subject table, simply click the field you wish to edit and type into it as you would a regular spreadsheet program. Pressing the "Enter" key will commit your changes to the table and advance the cursor to the next cell in the table as shown in Figure 10.

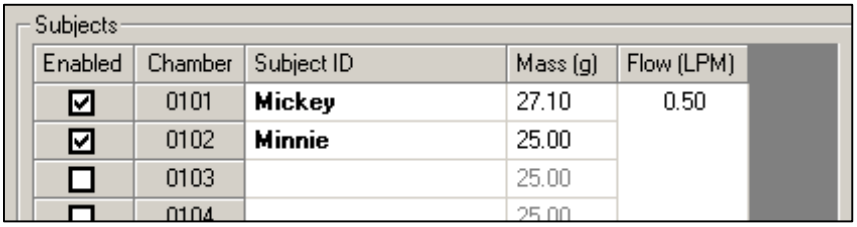


Figure 10 - Subject Setup

On the right-hand side of the Experiment Setup Window are a series of boxes where additional subject information can be stored with an experiment. The fields are not used during an experiment, but the information is attached to the selected subject and presented with the exported experiment data. The left column, "Label", can be used to define a series of properties for a subject (gender, color, age, etc) and the right column can store the values for those properties (male, brown, 30 weeks, etc).

The "Done" button commits any changes on this form to the experiment.

If the currently configured system features telemetry hardware, additional columns will appear in the table that allow for the assigning of E-Mitter transmitters to each chamber as in Figure 11.

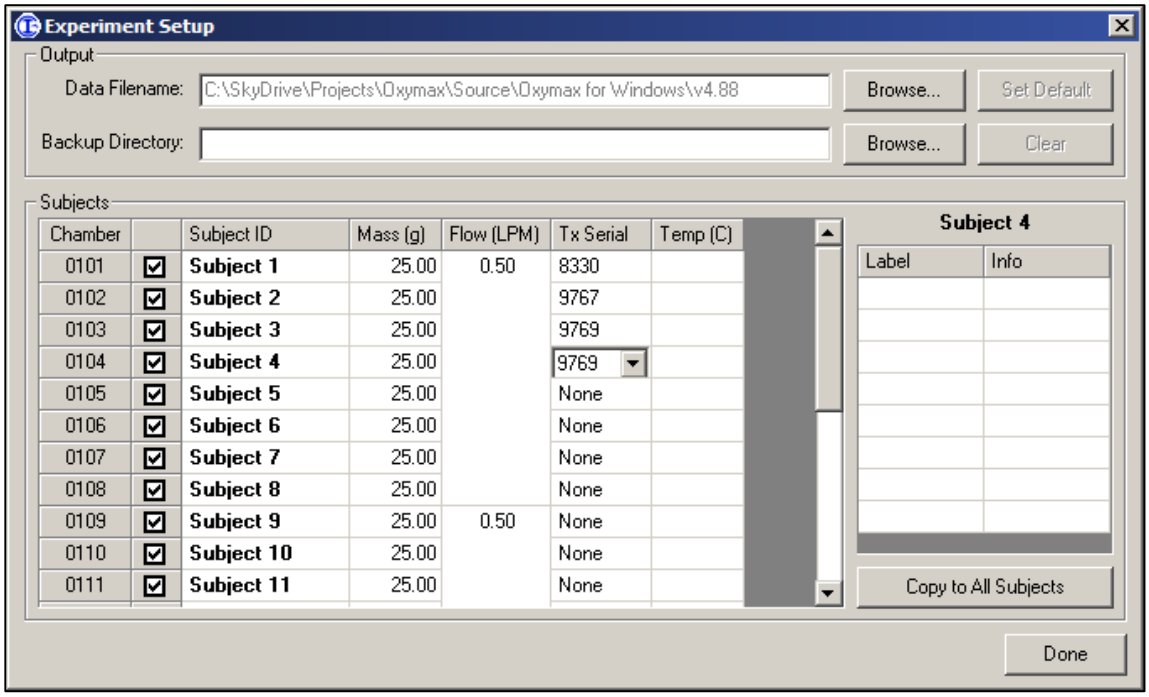


Figure 11 - E-Mitter Serial Selection

E-Mitter transmitters are serialized and have different factory calibration settings. Oxymax for Windows needs to know which transmitter is in each cage in order to properly interpret transmitted data. Each cell in this column is a drop-down list of "E-Mitter Transmitter" serial numbers. After implanting the transmitter in the subject (or allotting a transmitter to a particular subject), click the cell in the row for the chamber that subject will be tested in and select the serial number of the transmitter with which they were implanted. The columns to follow will begin to update with measurements after a serial number has been selected if the transmitter is within range or the receiver. The Heart Rate (HR) column will only appear if the system supports and is using transmitters equipped with heart rate leads.

3.3 Measurement Configuration

After the instance-specific settings for the experiment have been defined, you can use the Experiment Properties Window to customize the way in which the experiment is executed. The Experiment Properties Window is accessible from the "Experiment >> Properties" menu option. Not all options will be available to all users; settings make themselves available for systems that support them. Clicking "OK" at the bottom of the form commits any settings changes to the experiment.

By default, most altered settings are saved back onto the original Experiment Template (*.EXP). But, if you make changes to your experiment configuration that you wish to re-use, you can write them to the template so they become the default settings. To overwrite an existing set of Experiment Properties, just click "File >> Save Experiment Configuration". A few products store their settings in the devices themselves rather than any file and will persist between experiments regardless.

Note – Multiple Experiment Configurations

If you are consistently performing experiments with the same sets of different settings, you can use the "File >> Save Experiment Configuration As" menu option to save the current settings to a new file. When you wish to run that new experiment type later, just be sure to select it from the "Open Experiment Configuration" dialog when creating a new experiment.

3.3.1 Metabolic Properties

When the Experiment Properties Window first opens, it displays the settings for the metabolic components of the CLAMS system as in Figure 12.

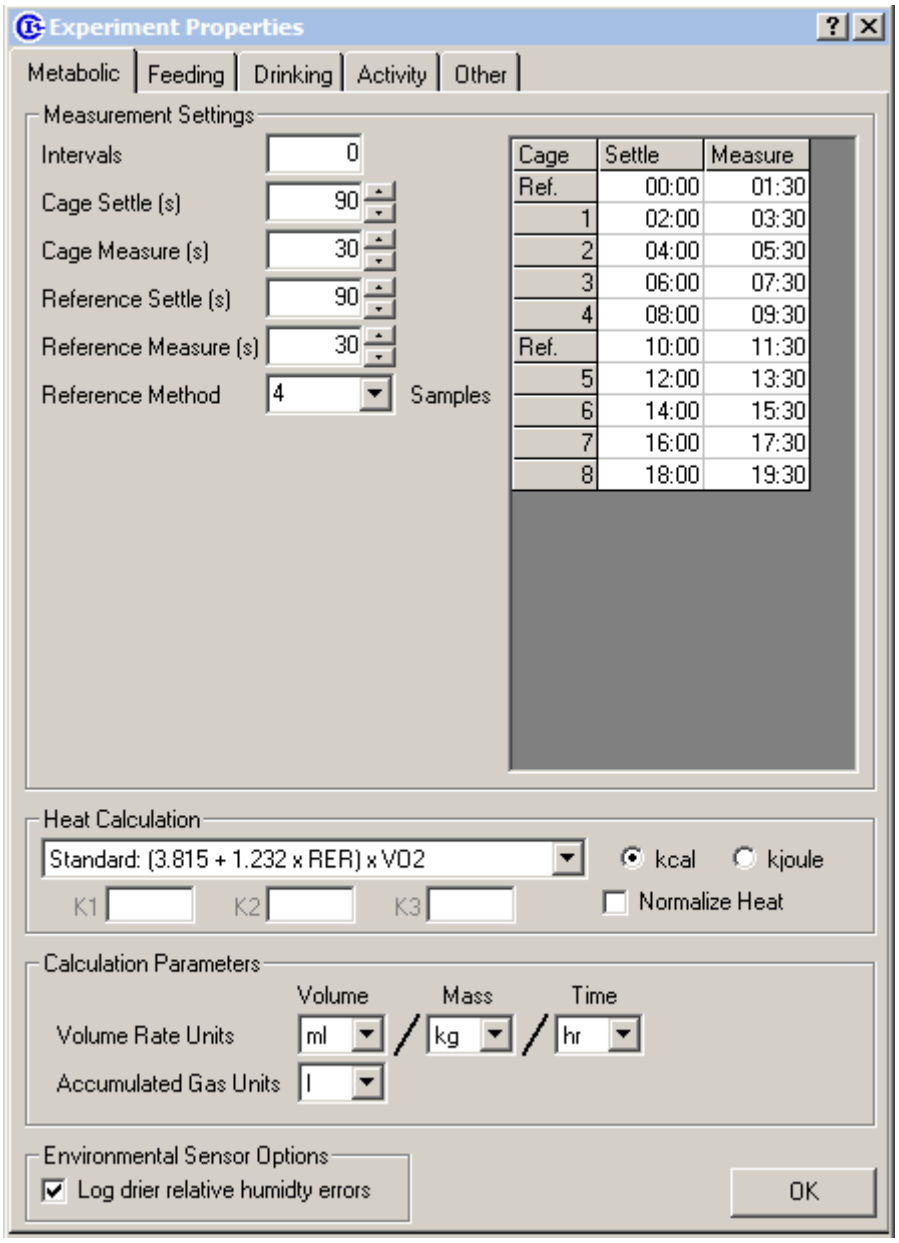


Figure 12 - Metabolic Experiment Properties

- **Measurement Settings** –These are all of the metabolic schedule and timing settings.
  - **Intervals** – The number of intervals to be recorded for the experiment. When set to "0" (zero), the experiment will run indefinitely until stopped by the user.
  - **Cage Settle** - The amount of time the system waits for the previously sampled gas to purge from the sensors when switching to a sample chamber.
  - **Cage Measure** – The amount of time the system measures the current sample. The gas sensors are sampled once every second for the specified duration and the average is recorded as the measurement. For non-metabolic CLAMS

systems, this value is used as the time in between samples for activity, drinking, urine, feeding and telemetry data.
    - **Reference Settle** – The amount of time the system waits for the previously sampled gas to purge from the sensors when switching to reference air.
    - **Reference Measure** – The amount of time the system measures reference air. The gas sensors are sampled once a second for the specified duration and the average is recorded as the "In" concentrations for measurements that follow the current reference (more on this in **5.1.1 Metabolic Measurements**).
    - **Reference Method** – This specifies the frequency of reference measurements during an experiment. A value of "4" (four) specifies that reference air will be measured after every four subjects. It is also possible to take only one reference measurement at the start of an experiment; this is not recommended and is only possible when the system is in a very environmentally stable room.
    - **Measurement Timeline** – The Measurement Timeline is the table on the right-side of the form that indicates the order and schedule for measurements under the current experiment settings. The times listed are presented in minutes since the start of the experiment or since the end of the last line of the schedule. The timeline will show multiple references or cage measurements up until the point the schedule repeats itself. The example in Figure 12 shows a system with four cages being referenced every four cages.
- **Heat Calculation** – By default, Oxymax for Windows uses a generally excepted equation for calculating energy expenditure based on metabolic measurements. Information on how this equation was empirically determined can be found in **Appendix A – Oxymax Calculations**. If you'd like to use a different equation for heat calculation, select "Custom" from the drop-down menu and specify coefficients for the volumetric figures: VO_2, VCO_2, and in the case of ruminant animals, VCH_4. You can also select the data to be presented in either kilojoules or kilocalories.
    - **Normalize Heat** – With this selected, the rate of heat expenditure be calculated normalized by initial animal body mass (energy / mass / time) using the same units as the gas exchange rate measurements.
- **Calculation Parameters** – These are the units that all of the metabolic calculations will be displayed in.
- **Environmental Sensor Options** – When enabled, logs humidity faults from the Environmental Sensor. Humidity readings from either the sample or the drier can indicate the drier is unable to dry the sample gas (out of descant, failing drier tubing, leak, etc).

3.3.2 Feeding Properties

Columbus Instruments offers a variety of options in regards to feed monitoring and control. Settings for those components appear on the "Feeding" tab of the Experiment Properties Window as in Figure 13.

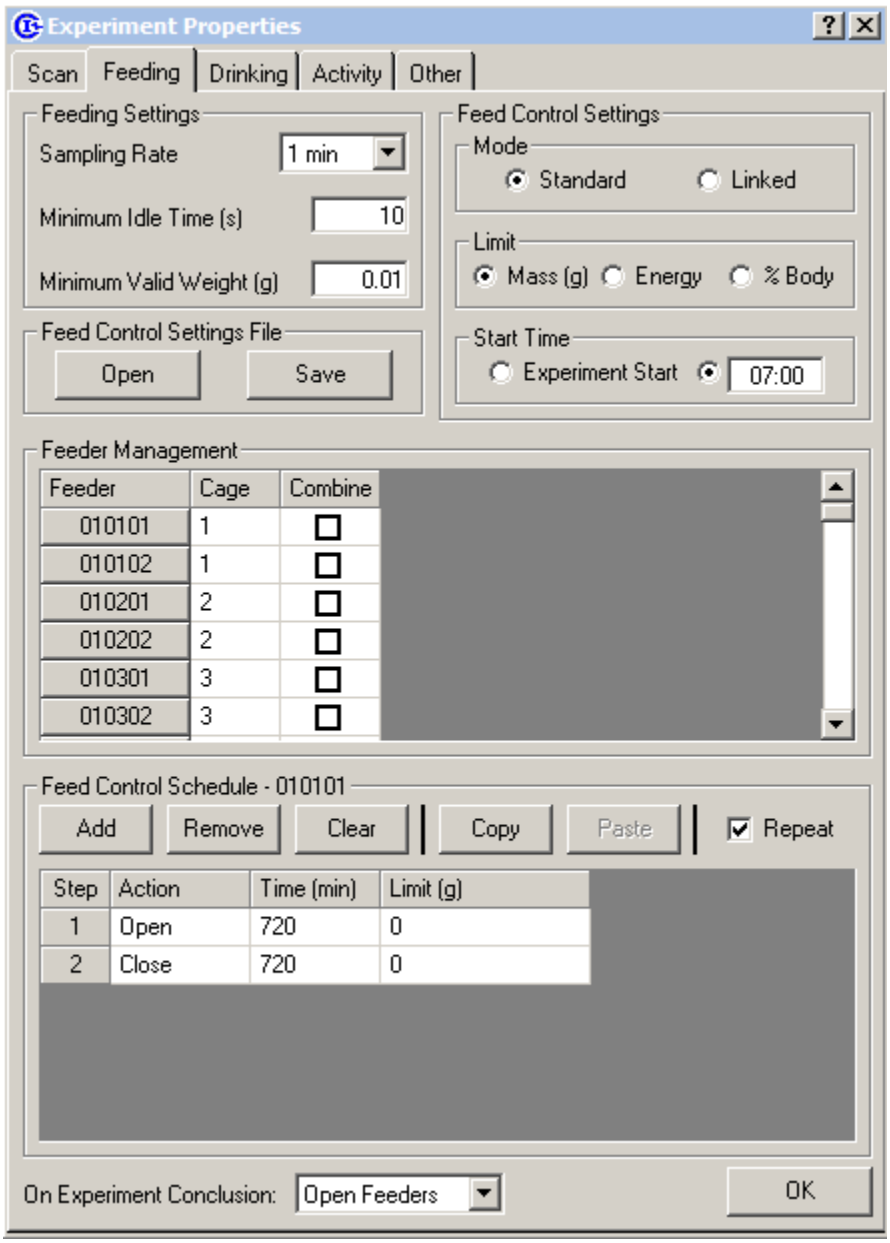


Figure 13 - Feeding Experiment Properties

- **Feeding Settings** –These settings are available for systems that feature any type of CLAMS feed monitor device (scale, strain gauge, etc).
  - **Feed Sampling Rate** – In addition to recording food consumption synchronously with metabolic data, all feeding devices can be sampled at a higher rate for higher resolution feeding data. The high resolution feeding data is stored separately from the metabolic data. Accessing and understanding this data is covered more thoroughly in **5.1.2 Feed Measurements**.
  - **Minimum Idle Time** – Oxymax for Windows can also record asynchronous, event-driven feeding data that meets specified conditions. "Minimum Idle Time" indicates the amount of time the animal must stop eating before the software

registers a completed feeding event. Understanding and using Feeding Event data is described in **5.1.2 Feed Measurements**.

- **Minimum Valid Weight** – This is the smallest amount of consumed food that the animal must eat in order to register a feeding event.

- **Feed Control Settings** – These settings define the operation of any attached feed control systems. The use of these settings is reasonably involved and covered completely in **3.4 Feed Control Settings**.

3.3.3 Drinking Properties

Columbus Instruments offers three options for measuring liquid consumption in CLAMS: lick counting, monitoring by mass, and volumetric drinking monitoring. The Volumetric Drinking Monitor (VDM) has a series of safety settings to warn the user of potential dehydration or malfunctioning sipper conditions. These warnings are referred to as "Under-Pump" and "Over-Pump" warnings.

Under-Pump errors are issued when the subject fails to drink a minimum amount of water over a given period of time. This can either happen because the animal is unable to access the sipper tube or the drinking system has malfunctioned in some way (clogged water line, empty water reservoir, etc). The warning is logged either when a subject fails to drink at all for a certain period of time (Under Delay) or the subject drinks less than a certain amount (Under Threshold) over a period of time (Under Period).

Over-Pump errors are issued if the drinking monitor pumps more water than is realistic for a subject to drink. Dirty sipper tubes can cause the system continuously pump water into the cage when the subject is not drinking. If the system pumps more than a certain number of droplets of water into the cage (Over Threshold) during a given interval (Over Period), then Oxymax for Windows can log an error and possibly disable the water pump for that channel (Over Disable).

The available drinking settings are depicted in Figure 14.

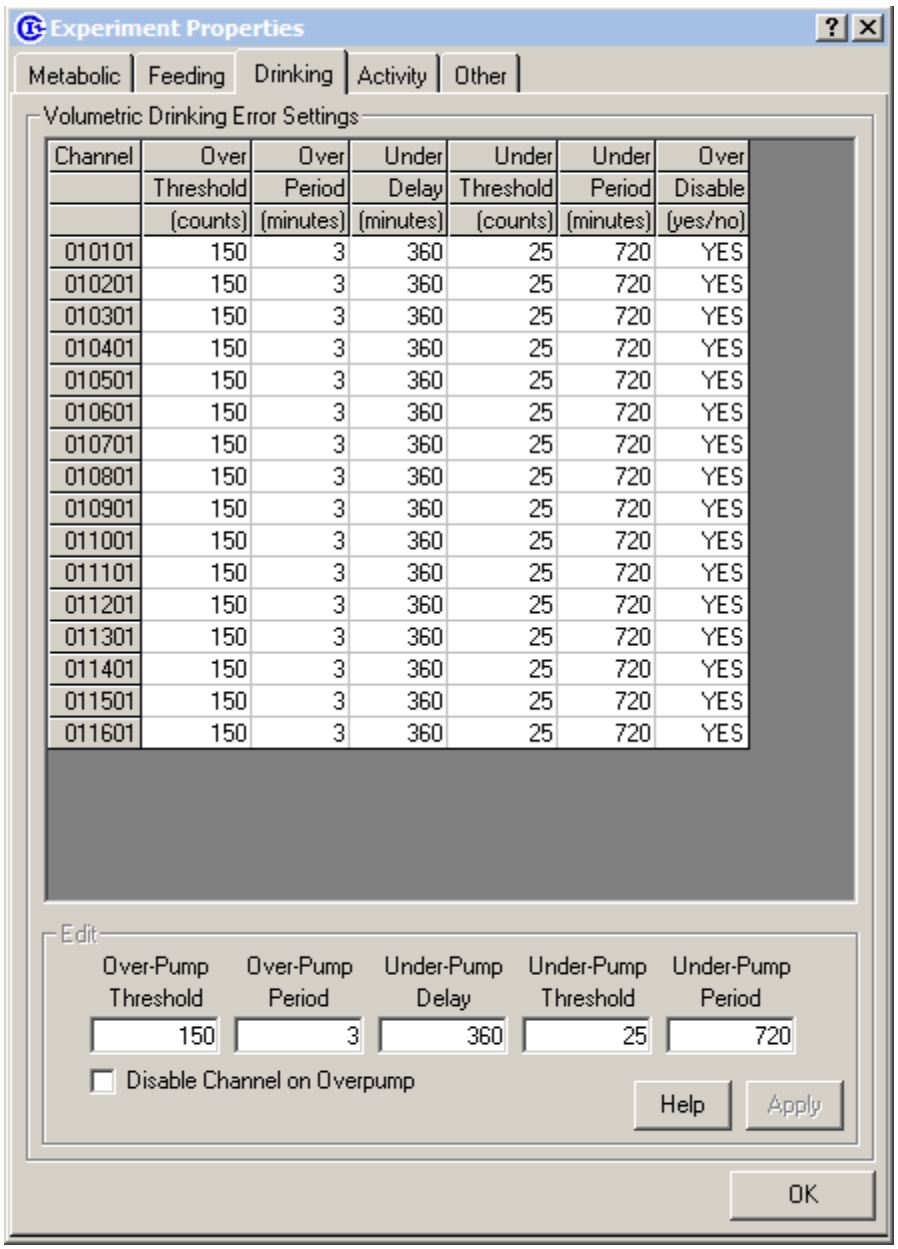


Figure 14 - Drinking Experiment Properties

- **Volumetric Drinking Error Settings** –This is the table of settings that control error handling for the Volumetric Drinking Monitor. The "Channel" column refers to a specific VDM sipper.
- **Edit** – To make changes to the settings table, select one or more VDM channels from the table and use the drop-down controls to change the settings for those channels. Hit "Apply" to commit those changes to the selected channels.

3.3.4 Activity Properties

Activity measurements are presented as the number of times the subject breaks an infrared beam during the course of a given interval. Chambers can monitor these beam breaks in up

to two axes just above the chamber floor and another axis near the ceiling used to measure rearing activity.

Animal activity can be recorded in a number of different ways by Oxymax. By default, Oxymax for Windows takes beam-break activity measurements at two frequencies: in-sync with the metabolic measurements and at a higher, independent rate. The high-frequency rate can be set by the user while the low-frequency rate is determined by the metabolic measurement time and number of enabled cages.

For systems that can measure beams breaks in both axes on the lower plane – the X and Y axes – Oxymax can also monitor animal position over the course of the

The activity settings for an experiment are presented in Figure 15.

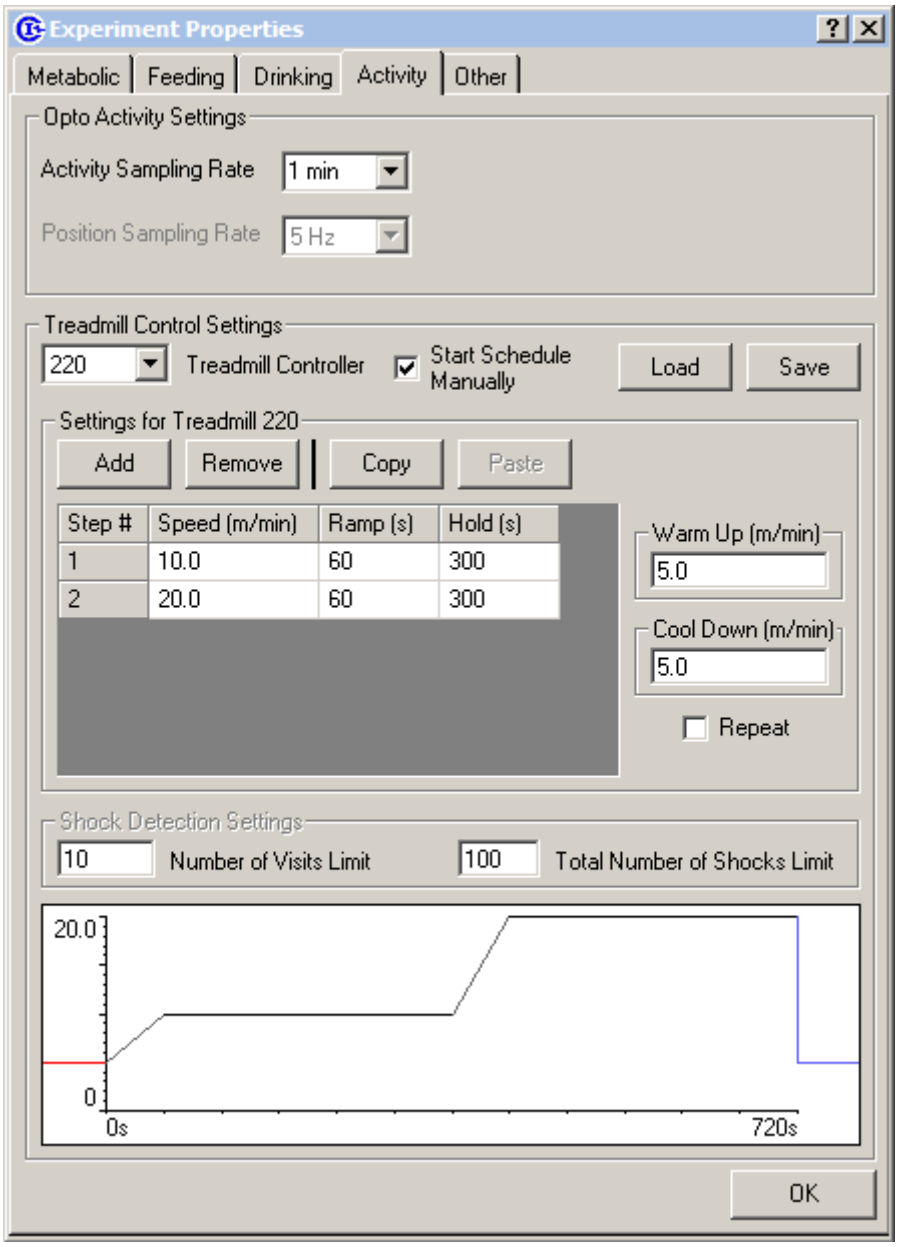


Figure 15 - Activity Experiment Properties

- **Activity Settings** – These settings are used in the acquiring and handling of data from the "Opto" family of beam-break activity sensors.
  - **Activity Sampling Rate** – The frequency in which high-resolution activity measurements are taken. This feature can also be disabled but it is not recommended.
  - **Position Sampling Rate** – The frequency in which the raw beam data from the activity monitors is read and stored by the software. This option is only available with systems using the Opto-M4 model of activity monitor and can be adjusted between 1 and 10 Hz.

- **Treadmill Control Settings** – These settings define the operation of any attached treadmill control systems. The use of these settings is reasonably involved and covered completely in **3.5 Treadmill Controller Settings**.

3.4 Feed Control Settings

Columbus Instruments' Feed Control system allows for independent or dependent control of access to every feeder in the system. Subjects or particular diets within a subject's chamber can be set to follow unique schedules or cyclical patterns. The system can be run in one of two modes: Standard or Linked. Consumption limits for subjects can be specified in grams, energy consumed or consumption as a percentage of animal body mass. The Feed Control settings pictured in Figure 13 only appear if the system is equipped with a Columbus Instruments FeederMax control system.

The "Feed Control Settings" frame in the corner of the Feeding tab allows for the selection of basic control settings. Custom control schedules can be saved and loaded from the "Feed Control Settings File" frame. The "Balance Management" table displays all balances for every enabled chamber in the system along with mode-specific settings. The "Feed Control Schedule" table displays the schedule for the currently selected balance or group of balances from the Balance Management table.

3.4.1 Standard Feeding

The Standard feeding mode allows for each feeder to be controlled independent of the other feeders in the system. The exception to this independence is in cases where subjects have access to multiple feeders. Feeders in the same chamber have the option of being combined to share limits and access conditions.

Under the "Balance Management" table, clicking the ID in the left-most column for a feeder displays the schedule for that feeder in the "Feed Control Schedule" table as in Figure 16. The system defined in Figure 16 features two feeders in each cage and therefore displays an additional setting labeled "Combine".

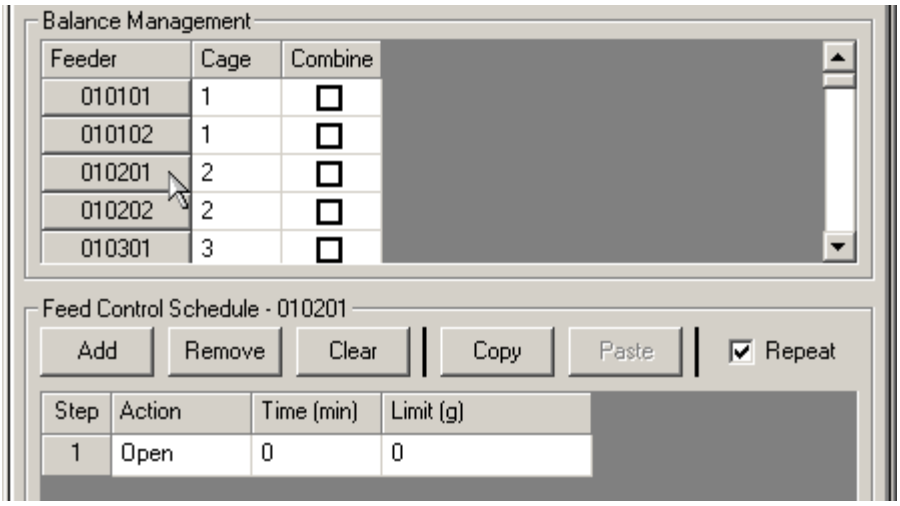


Figure 16 - Selecting a Balance

Each balance has a schedule comprised of one or more "steps". Each step is comprised of an action, a time and a limit. The action (either "Open" or "Close") is performed at the start of the step which lasts until either the time or limit condition is reached. A time limit of zero indicates that the feeder will remain at its current state for an indeterminate amount of time. A limit of zero denotes that the subject can consume any amount of food during the current step. A step with no time or mass limit will last for the duration of the experiment; the schedule in Figure 16 allows the animal access to food for the entire experiment.

Click the "Add" button above the Feed Control Schedule table to add new steps to the current schedule. The "Remove" button deletes the last step in the schedule while "Clear" removes all but the first step. The "Repeat" checkbox in the corner of the schedule indicates whether or not the current schedule will automatically return to the first step at the end of the last step.

A simple day and night feeding schedule would consist of alternating 12 hour feeding and 12 hour fasting periods. Figure 17 shows a limitless, repeating feeding cycle for a single balance.

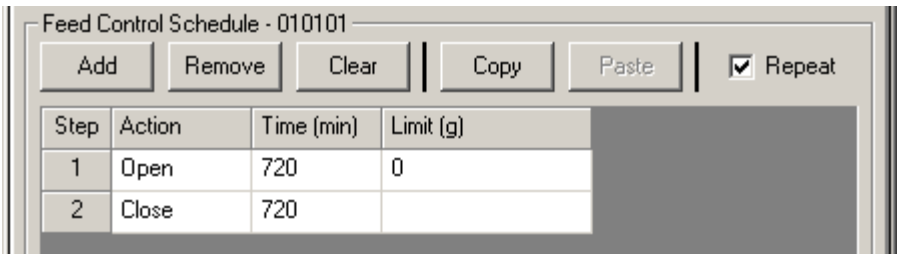


Figure 17 - Simple Day/Night schedule

- **Step 1** – Opens the feeder and leaves it open for 720 minutes (12 hours).
- **Step 2** – Closes the feeder and leaves it closed for 720 minutes.
- **Repeat** – Returns the feeder to Step 1.

A limit can be placed on an "Open" step that causes either the feeder to close or the controller to advance to the next step when reached. If the step has a time limit and a consumption limit, the feeder will close for the remainder of the specified time when the consumption limit is reached. If a consumption limit is specified without a time limit, the controller will advance to the next step when the subject reaches the specified limit. Figure 18 shows a modified version of the day/night schedule from Figure 17 with the addition of limits.

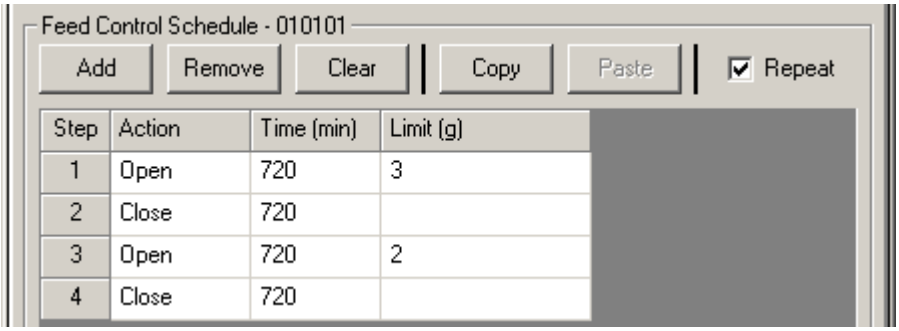


Figure 18 - Day/Night Schedule with Limits

- **Step 1** – Opens the feeder and leaves it open for 12 hours or until the subject consumes 3 grams of food. The controller remains in this step for the entire 12 hours regardless if the consumption limit is met or not.
- **Step 2** – Closes the feeder for 12 hours.
- **Step 3** – Opens the feeder and leaves it open for 12 hours or until the subject consumes 2 grams of food. The controller remains in this step for the entire 12 hours regardless if the consumption limit is met or not.
- **Step 4** – Closes the feeder for 12 hours.
- **Repeat** – Returns the feeder to Step 1.

This schedule allows the animal to eat up to 3 grams for 12 hours during the first day and 2 grams during the same 12 hours on the next day. This pattern repeats for the duration of the experiment.

Systems with multiple feeders in each chamber have the option of combining those balances together and are treated like one feeder. Combined balances share feeding schedules and the limits in those schedules are evaluated using the sum of consumption from all combined balances in the same chamber.

3.4.2 Linked Feeding

The Linked feeding mode allows for subjects' access to food to be limited by the consumption of other subjects. Linked feeding can be set up to perform "paired" feeding experiments. Within Linked feeding experiments, feeders are placed into different groups and are assigned as masters or slaves within that group. One or more masters within a group control the amount of food the slaves within that group can consume.

Clicking the "Linked" option button under the "Feed Control Settings" frame inserts additional settings to the Balance Management table: "Group", "Master" and "Multiplier". Balances can be assigned to any group between 1 and 128. There is no limit on the number of slaves or masters in any given group. Figure 19 shows an example experiment where balances are placed into groups of four with two masters and two slaves in each group.

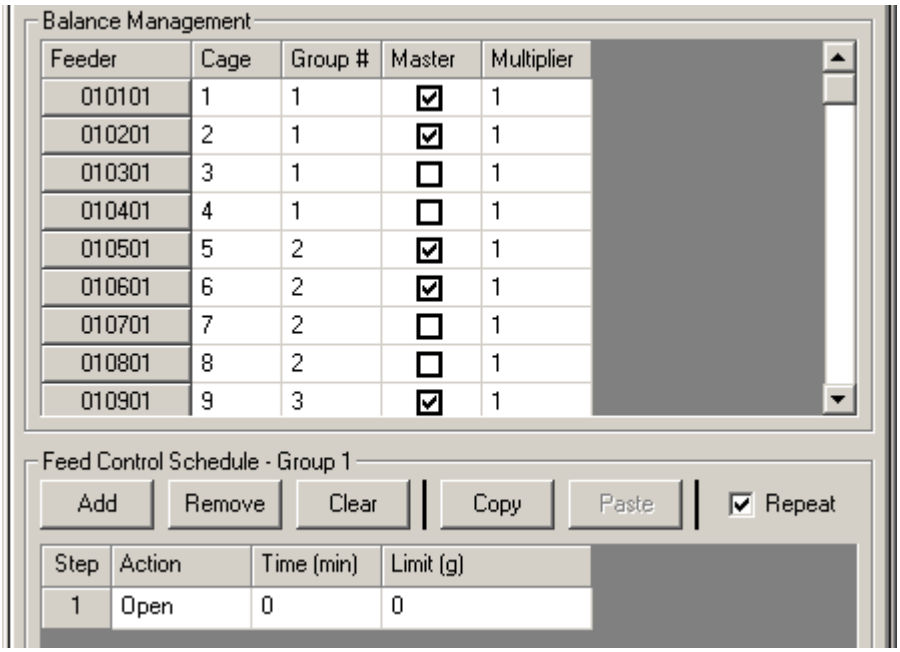


Figure 19 - Linked Feeding Experiment

When performing a linked feeding experiment, schedules are specified for by all group masters rather than each individual balance. All masters within a group share the same schedule. According to Figure 19, the masters for Group 1 are permanently open for the duration of the experiment.

At any given moment, the slaves from Group 1 are allowed to have consumed as much food as the average consumption of the masters from Group 1. If a slave's consumption equals or exceeds the masters' average consumption, its feeder is closed and remains closed until the masters' average increases.

For example, Table 1 represents the consumption and feeder statuses of all subjects in Group 1 (as defined in Figure 19) a few hours after the start of an experiment.

Balance	Consumed	Controller
010101	3.00	Open
010201	1.00	Open
010301	2.10	Closed
010401	2.20	Closed

Table 1- Group 1 Consumption Example

In this case, the masters from Group 1 have eaten an average of 2.00 grams each (3.00 grams + 1.00 grams / 2 masters). The slaves both ate to that limit and slightly above. The system cannot immediately restrict access to food when a slave passes a limit because the balance has to stabilize for a second before Oxymax can determine if the limit has been reached. If the subject using Balance 010201 consumed another quarter of a gram of food, the average for the masters would rise to 2.125 grams. The consumption on Balance 010301 would then be below the masters' average and the feeder would open as illustrated in Table 2.

Balance	Consumed	Controller
010101	3.00	Open
010201	1.25	Open
010301	2.10	Open
010401	2.20	Closed

Table 2 - Group 1 Consumption Example, continued

3.4.3 Feeding Limits

Consumption limits are specified in grams by default but can also be stipulated by energy consumption or consumption as a percent of subject body mass. Selecting "Energy" from the "Limit" options as in Figure 20 adds an additional setting to the Balance Management table: "Energy/g".

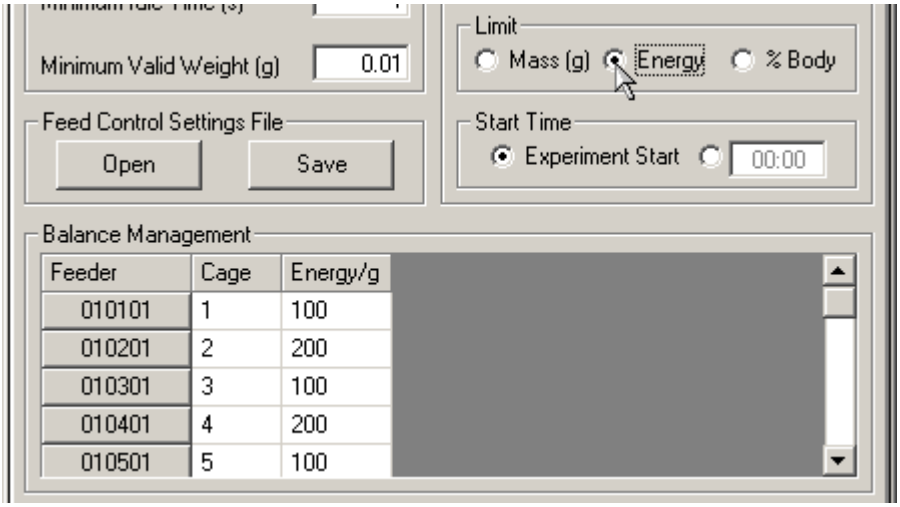


Figure 20 - Feeders with Two Different Diets

Figure 20 shows that the feeders in this experiment are filled with two diets with different calorific content.

> **Note – Energy has no units**
>
> The term "energy" is used instead of a specific unit for energy. Energy concentrations for diets can be specified as any unit as long as the same unit is used consistently.

Energy limits can be used on systems with multiple feeders per subject to run studies involving diet preference while limiting consumption based on calories. The settings pictured in Figure 21 illustrate how to restrict total consumption by a subject while still allowing for it to make choices about its diet.

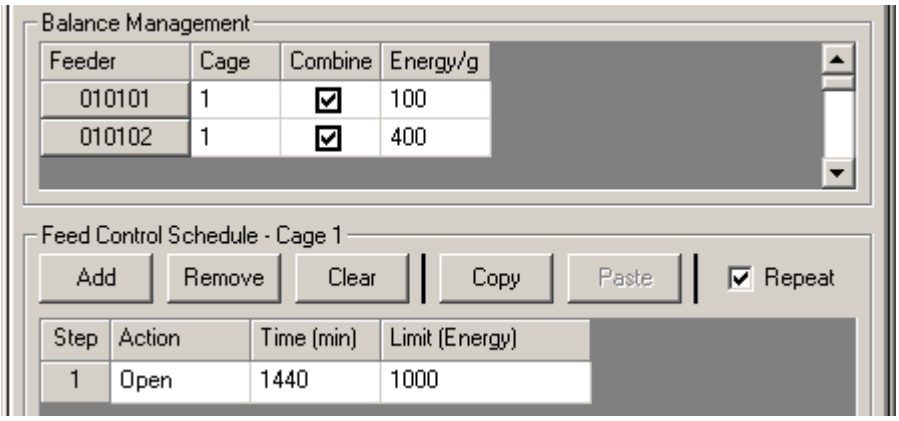


Figure 21 - Feeding Preference with Limits

In this case, the subject in Cage 1 would be permitted to consume a total of 1000 "energy" every 24 hours. Because the balances for this subject have been combined, the limit defined in the schedule applies to the sum of both balances. The subject could consume 10 grams of food from the first feeder, 2.5 grams from the second or any combination of therein. When the subject reaches the limit, both feeders in the chamber will close.

Specifying limits by body mass may be used when trying to normalize consumption across subjects of varying size. Limits specified by body mass use the subject's pre-experiment body mass as specified on the Experiment Setup window (see **3.2 Experiment Setup**). The schedule shown in Figure 22 would dictate that the selected subject is permitted to eat 5% of its body weight every 12 hours.

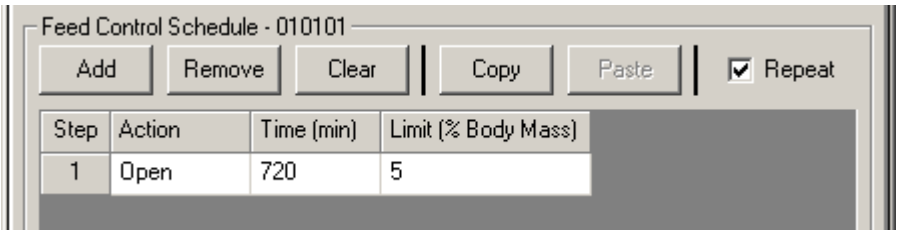


Figure 22 - Limiting Consumption by Body Mass

Linked experiments have an additional setting related to consumption limits called "Multiplier". By default, this value is "1" indicating that consumption is multiplied by one before used in master/slave calculations. For example, setting the multiplier to "0.75" for a slave would mean that the subject using the slave feeder would be permitted to eat 75% of its masters' average before the feeder would close. Conversely, raising or lowering the multiplier for a master feeder effectively "weights" that feeder's contributions to its group's

average. If a master feeder has 2 grams of consumption and a multiplier of 1.5, its contribution to the group's master average is 3 grams.

3.4.4 Feed Control Scheduling

Feed Control schedules can be set to start at the beginning of an experiment or at a specific time of day. Selecting "Experiment Start" from the "Start Time" options starts all enabled feeders in the experiment to Step 1 when the experiment starts. Selecting the "Start Time" option allows for the selection of a particular time in which to activate the first step of each feeder's schedule. The selected time can either be in the future or the past, relative to the time the experiment is started.

Selecting a start time that is earlier than the current time will cause Oxymax to calculate what step the feeder would be on if the experiment had actually been started at the specified time. For example, if the day and night schedule specified by the settings in Figure 17 was set to start at "07:00" (7:00 am) and the experiment was started at 1:00 pm, the feeders would open at the start of the experiment and remain open until 7:00 pm. Even though the feeders would only have actually been open for 6 hours, the schedule proceeds as if it had actually been started at the specified time.

If the selected schedule start time is later than the experiment start time, all feeders remain in the current state until the specified time.

3.4.5 Duplicating Schedules

Complicated or frequently used schedules can be copied from one feeder and applied to others. Clicking the "Copy" button above the currently selected Feed Control Schedule will put that current schedule onto the clipboard for use on other feeders. Select another feeder and click "Paste" to replace that feeder's current schedule with the one on the clipboard. When configuring a linked experiment, schedules cannot be pasted onto slave feeders.

3.4.6 Saving and Opening Feed Control Settings

After a set of feeding schedules for an experiment has been created, those settings can be saved to the hard disk for use on other experiments. This is recommended for any group of feed control settings that will be used frequently.

To save the current settings to file, click "Save" under the "Feed Control Settings File" heading. The dialog window like one pictured in Figure 23 - Save Feed Control Settings will appear where the name and location of the ".FCTL" (Feed Control) file can be specified.

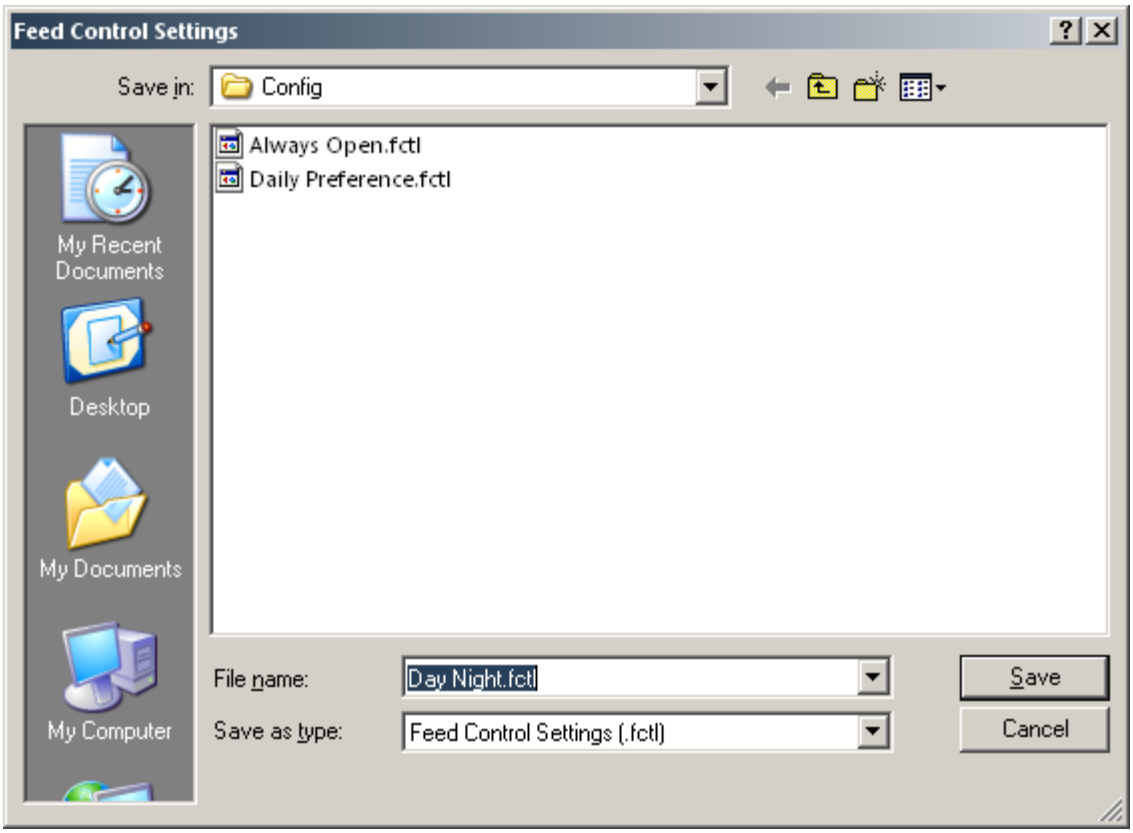


Figure 23 - Save Feed Control Settings

It's important for users with access to multiple CLAMS systems to note that Feed Control Settings files can only be used with the system for which they were created.

3.5 Treadmill Controller Settings

Columbus Instruments' Treadmill Controller allows for the scheduled control of one or more lanes of a metabolic treadmill during an Oxymax experiment. Oxymax can be used to set customized speed schedules for treadmills while simultaneously collecting metabolic data. The Treadmill Control settings pictured in Figure 15 only appear if the hardware configuration file in use contains settings for a Treadmill Controller device. Many systems that make use of metabolic treadmills are also equipped with regular chambers and usually ship with separate hardware configurations for the two setups.

Note – Check the Treadmill Controller for Oxymax Compatibility

Not all Columbus Instruments Treadmill Controllers support Oxymax integration out of the box. Older Treadmill Control units may require a firmware update and modifications to the associated hardware configuration file. Contact Columbus Instruments to discuss Treadmill Controller options.

3.5.1 Basic Settings

A custom treadmill schedule consists of three parts: the warm-up period, the schedule itself and the cool-down period. When Oxymax initializes the specified hardware upon startup, it sets the treadmill speed to the last used "warm-up" speed which will be maintained until the start of the experiment schedule.

> **Note – Computer Control of the Treadmill Controller**
>
> Please note that in order for Oxymax to adjust the treadmill belt speed the Treadmill Controller must be set into either the "Run" or "Accel" position on the front panel. Switching the Treadmill Controller to "Stop" at any point immediately stops the Treadmill. After the software has assumed control of the unit the Treadmill Controller's controls for speed and electric stimulus will be disabled until the controller is "Reset" with the button in the lower-left corner of the front panel.

Figure 24 shows an example of a treadmill experiment with some standard settings applied.

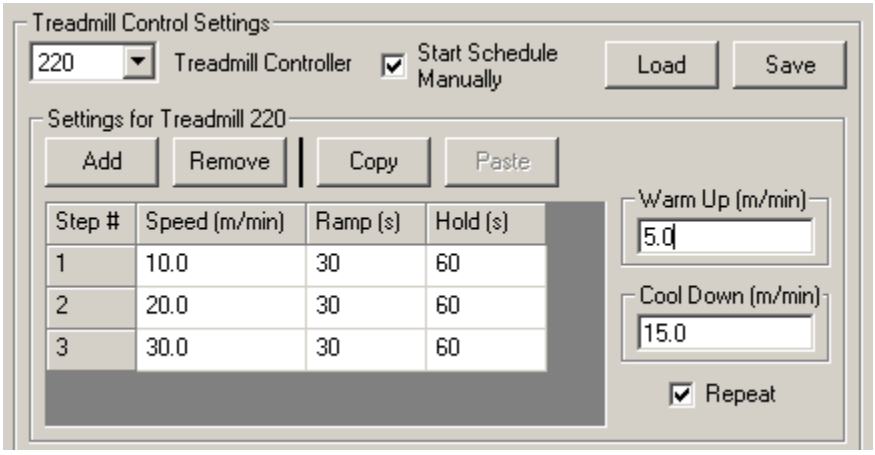


Figure 24 - Basic Treadmill Settings

- **Selected Treadmill Controller** –This drop-down box contains a list of all of the Treadmill Controller units in the current hardware configuration. Selecting a device address from this list displays the current settings for that Treadmill Controller.
- **Load / Save** – Allows for the saving and reloading of frequently used Treadmill schedules as ".tread" files.
- **Start Schedule Manually** – When this option is checked, Oxymax will not immediately start the specified exercise schedule at the beginning of the experiment but rather wait for the schedule to be started from the "Start Treadmill Schedule" option in the "Experiment" menu.
- **Schedule Table** – This table details the stages of the Treadmill Control schedule to be executed upon the schedule's start. Details for specifying treadmill schedules are further explained in **3.5.2 Treadmill Schedules**.
- **Repeat** – When checked, this will cause the specified treadmill schedule to continuously repeat for the duration of the experiment.

- **Warm Up** – The speed the treadmill will be set to upon initialization and continue to use until the start of the treadmill schedule. Changes to this value will be immediately applied to the selected Treadmill Controller.
- **Cool Down** – The speed the treadmill will be set to at the conclusion of either the treadmill schedule or the experiment itself. Usually, this is the same speed as the warm-up speed.
- **Schedule Distance** – The distance the treadmill will travel by the end of one complete iteration of the defined treadmill schedule. This value is updated when any of the schedule parameters are changed.

3.5.2 Treadmill Schedules

The treadmill schedule itself is configured much in the same way as a feed control schedule. When the schedule starts, Oxymax progresses each treadmill through the series of defined steps; each of which consist of three properties:

- **Speed (m/min)** – The target speed that the treadmill will reach by the end of the step. The selected speed is range-checked against the limitations of the treadmill motor defined in the Hardware Configuration file.
- **Ramp (sec)** – The period of time during which the treadmill will accelerate to the specified speed. The acceleration rate is dependent on the "ramp" time as well as the previous speed of the treadmill. When the ramp time is set to zero, the Treadmill Controller will proceed to the requested speed as fast as mechanically possible.
- **Hold (sec)** – The amount of time the treadmill will remain at the requested speed before proceeding to the next step in the schedule. When the hold time is set to zero, the schedule will proceed to the next step as soon as the treadmill has reached the target speed.

The buttons above the schedule table as shown in Figure 25 can be used to make changes to the currently selected treadmill's schedule.

- **Add** – Adds a new step the current treadmill schedule.
- **Remove** – Removes the last step from the current treadmill schedule.
- **Copy** – Copies the current treadmill schedule to the clipboard for pasting on other Treadmill Controllers.
- **Paste** – Pastes the settings from the Treadmill Controller on the clipboard to the currently selected controller.

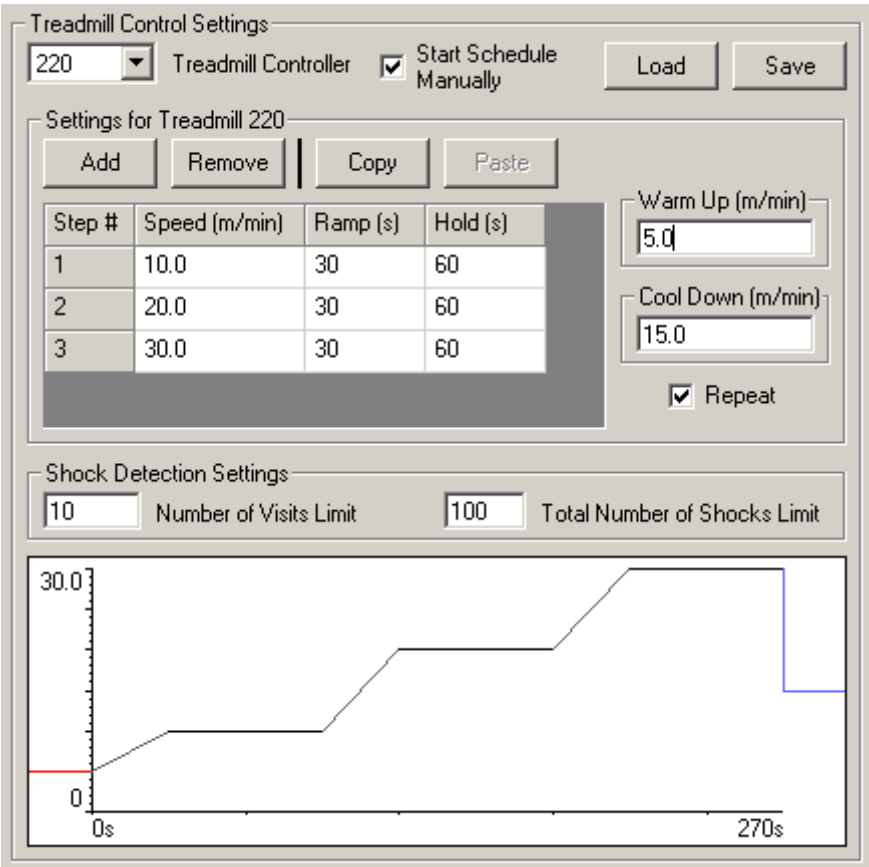


Figure 25 - Treadmill Schedule

To make changes to the treadmill schedule, select the cell in the table and use the keyboard to change the desired value. Press the "Enter" key to commit the altered value to the schedule.

The current schedule is depicted in the timeline beneath the schedule shown in Figure 25. The red line indicates the speed of the treadmill during the warm-up period; the line in blue represents the cool-down period. The plot will automatically adjust the scale to contain the entire length of the schedule as well as the schedule's highest speed.

3.5.3 Treadmill Shock Detectors

Also depicted in Figure 25 are settings for the optional Treadmill Shock Detection product. When combined with a Treadmill Controller, the Treadmill Shock Detection device can record shocks and shock events as well as disable the shock grids when the subject exceeds customizable limits.

- **Number of Visits Limit** – This value represents the total number of visits to the shock grid a subject can make before the grid is disabled. A "shock visit" begins on a first shock and concludes when the animal leaves the shock grid. This value is adjustable from between 1 and 999 visits.

- **Total Number of Shocks Limit** – This value represents the total number of shocks a subject will receive before the shock grid is disabled. This vale is adjustable from between 1 and 999 shocks.

3.5.4 Performing Treadmill Experiments

After schedules have been specified for all connected Treadmill Controllers the rest of the Oxymax experiment can be configured as it normally would. When the experiment is started, Oxymax will again check that the switch on the front of the Treadmill Controller to "Run" or "Accel" so that it can take control of the treadmill motor.

If the "Start Schedule Manually" option was not selected before the experiment begins the Treadmill Controller will immediately proceed to the first step of the schedule. If the manual-start option was selected, the treadmill will continue at the warm-up pace until the schedule is started by the user. The option to start the treadmill schedule appears in the "Experiment" menu as "Start Experiment Schedule".

Schedules set to repeat will continue to repeat until the experiment is stopped or the treadmill schedule specifically is stopped. Any running treadmill schedule can be stopped without halting an experiment with the "Stop Experiment Schedule" menu option under the "Experiment" menu.

Whether the treadmill schedule is stopped manually or with the conclusion of the experiment, the treadmill will proceed to the cool-down speed specified in the settings. When the Oxymax for Windows program is closed, the Treadmill Controller will immediately stop the motor. At this point, the controls on the front of the Treadmill Controller will remain disabled until the black "Reset" button in the lower-left corner of the front panel is pushed.

3.6 Environmental Controller Settings

Newer Environmental Enclosures may be equipped with Columbus Instruments' Environmental Controllers which allow for the control and recording of the lighting and temperature in the enclosure. The color and intensity of the lighting can be set for both Light and Dark intervals with customizable sunrise and sunset periods. The temperature set-point can be adjusted between 2 and 50 degrees Celsius with gradual adjustments that can span days or weeks. Both temperature and lighting schedules can be set on a by-system or by-controller basis. The "Selected Controller" dropdown box lists all Environmental Controllers and the chambers they contain. Selecting an Environmental Controller will update the window with that unit's current settings.

> **Note – Environmental Controller Retrofitting**
>
> Some Environmental Enclosures that shipped before 2014 are still capable of having an Environmental Controller unit installed on them for control of *temperature only* as they already have incompatible fluorescent lighting installed on them. Contact Columbus Instruments for information on adding Temperature-only control to existing units.

3.6.1 Temperature Control Scheduling

Figure 26 shows the options for temperature control on an Environmental Controller.

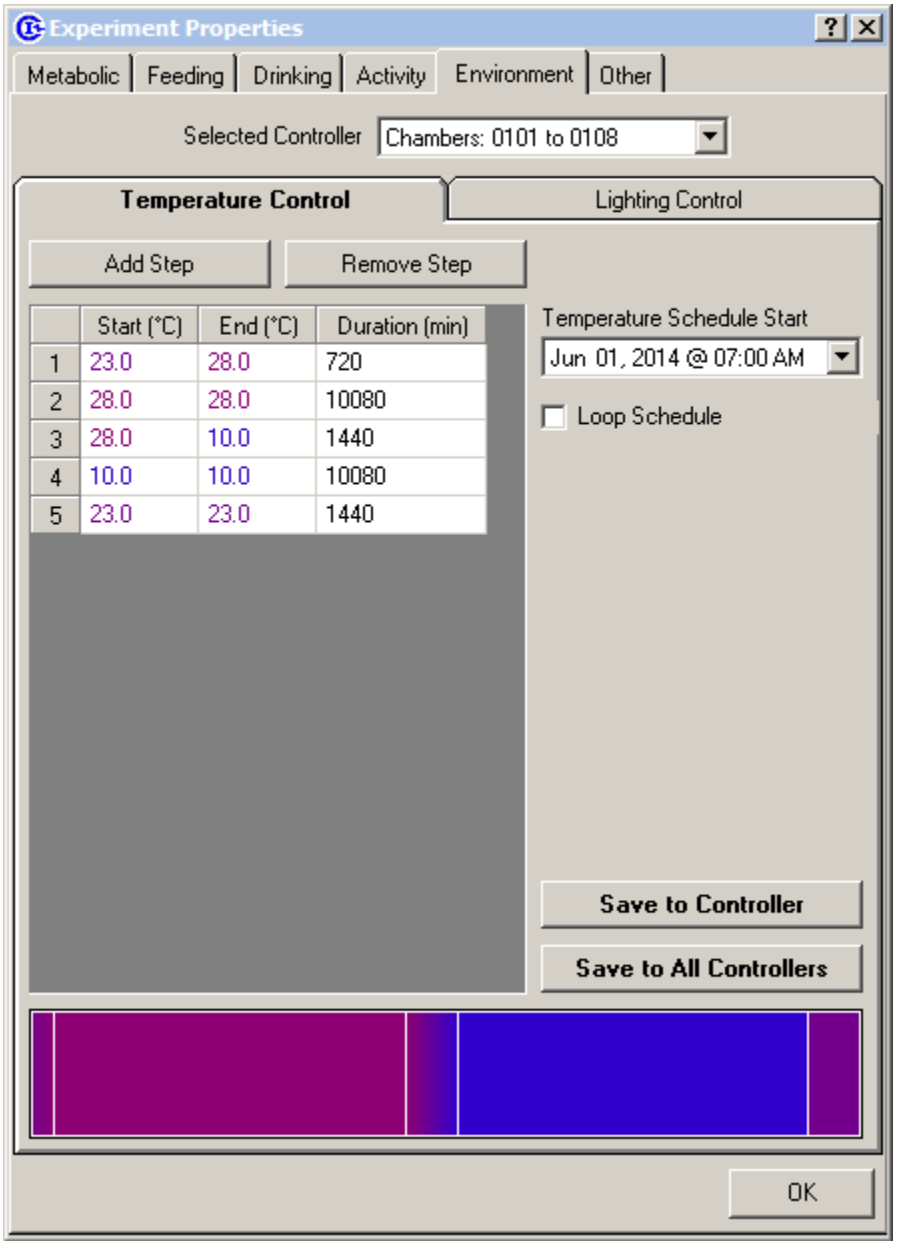


Figure 26 - Environmental Controller Temperature Schedule

Temperature schedules can have up to 32 individual steps with each step comprising of a starting temperature, ending temperature and duration to transition between those temperatures. The temperatures specified are *set-point* temperatures and may not reflect the immediate temperature in the enclosure. The time to achieve target temperatures may vary based on the size of the enclosure and other atmospheric conditions.

- **Add/Remove Step** – Adds/Removes a step to/from the end of the temperature schedule.
- **Start** – The temperature set-point at the start of the step.
- **End** –The temperature set-point at the end of the step. If the start and end temperatures are different, the controller will adjust the temperature linearly across the duration of the step.
- **Duration** – If the set-point temperatures are the same, this is the amount of time the controller will hold a constant temperature. If they are different, this is the period in which the temperature will change from Start to End. This value is expressed in minutes and can set to any value between 1 minute and 60 days (86400 minutes).
- **Temperature Schedule Start** – The date and time that the defined temperature schedule begins. If the specified time is in the past, the controller will immediately shift to the appropriate step in the schedule upon saving the schedule to the unit. If the specified start time is set in the future, the controller will remain at the current set-point until the schedule begins.
- **Loop Schedule** – When checked, the specified schedule will loop back to the first step when the last step is completed. When unchecked, the controller will remain at the End temperature of the last step.
- **Save to Controller** – Saves the specified settings to the selected Environmental Controller.
- **Save to All Controllers** – Saves the current settings to all enabled Environmental Controllers.

The bottom of the Temperature Control settings pane contains a timeline that show the currently listed schedule with bright red representing higher temperatures and blue representing lower temperatures. Each step in the schedule is bordered in white.

> **Manual Control**
>
> Removing every step from the Temperature schedule will release the Watlow controller on the enclosure to manual control. Oxymax will still record actual and set-point temperatures.

3.6.2 Lighting Control Scheduling

Figure 27 shows the settings available for lighting scheduling on the Environmental Controller.

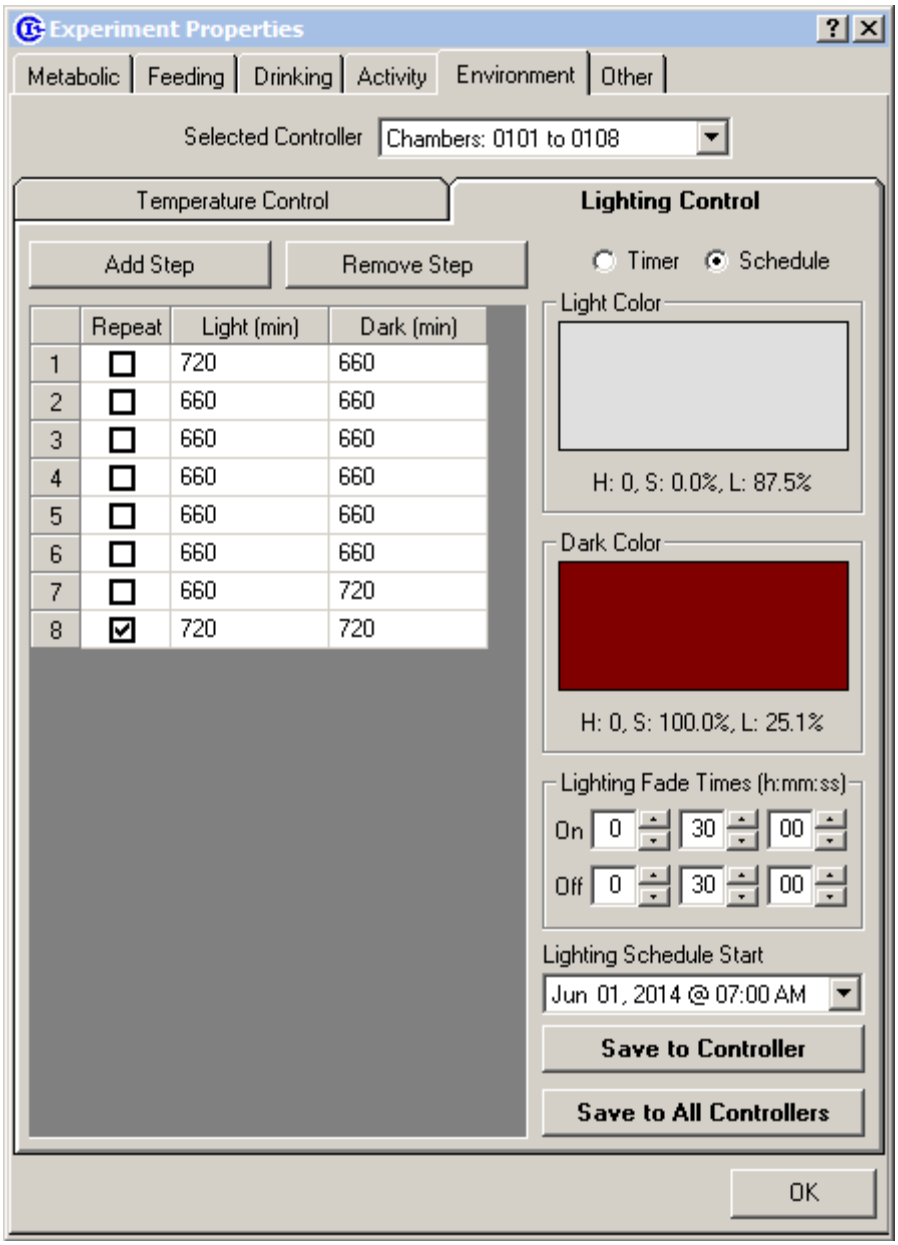


Figure 27 - Environmental Controller Lighting Schedule

Lighting schedules can have up to 32 steps that consist of a Light interval and a Dark interval. Each interval can be set up to 18 hours and as low as zero minutes. The colors that are used on the LED – Light Emitting Diode – light strips for Light and Dark can be customized in both hue and intensity.

- **Timer/Schedule** – Allows the lighting to be controlled either by the external Timer on the Environmental Enclosure or scheduled explicitly by the Environmental Controller.
- **Add/Remove Step** – Adds/Removes a step to/from the end of the lighting schedule.

- **Repeat** – When the schedule reaches the last step, the controller will loop back around to the step marked "Repeat". This allows for an "acclimation period" of one or more steps followed then by a repetitive pattern of one or more steps.
- **Light/Dark (min)** – The intervals in which the lights will be on and off in each step. Customizable between zero minutes and 18 hours.
- **Light/Dark Color** – Clicking either color panel will bring open the Windows Color Selector prompt where a color for each state can be selected. Colors on the LED's will not appear exactly as in the software; it's suggested that both colors are verified before running an experiment to make sure they match expectations. Information on each color is presented in standard HSL – Hue, Saturation, Lightness – format.
- **Lighting Fade Times** – The LED lighting can be set to fade from Light to Dark and Dark to Light over customized periods up to 18 hours. This can be used to simulate sunrise and sunset in the enclosures.
- **Lighting Schedule Start** – If the Lighting Controller is set to "Schedule" mode, this is the time that the schedule will begin its first "Light" step. As with the Temperature Schedule, if the time is set in the past the controller will automatically shift to the appropriate step the current schedule. If set in the future, the lights will remain at their current state until the schedule begins.
- **Save to Controller** – Saves the currently displayed Lighting settings to the selected Environmental Controller.
- **Save to All Controllers** – Saves the current displayed Lighting settings to all Environmental Controllers.

3.7 Other Settings

Settings for smaller sub-systems of CLAMS can be found under the "Other" tab in Experiment Properties.

3.7.1 Telemetry

Oxymax for Windows allows for a filter to be applied to heart rate data acquired through the optional telemetry system as seen in Figure 28.

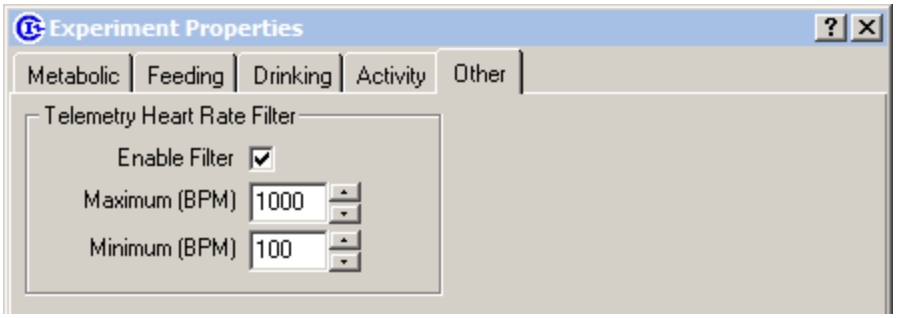


Figure 28 - Telemetry Settings

When enabled, heart rates outside the specified range will not be included in the averages presented in the data. Be careful when using this filter as data outside the specified range cannot be recovered.

3.7.2 Urine Chillers

Systems equipped with the Waste Management bases have an available Urine Chiller option. The chillers can be configured to automatically freeze and thaw urine and at the start and end of an experiment with the settings shown in Figure 29.

Selecting “Enable Chillers” will give control of all units over to the software which will begin freezing the sample vial at the start of an experiment. “Auto-Thaw” indicates that the chillers will automatically warm the sample for the indicated amount of time (selectable between 1 and 5 minutes) at the conclusion of an experiment.

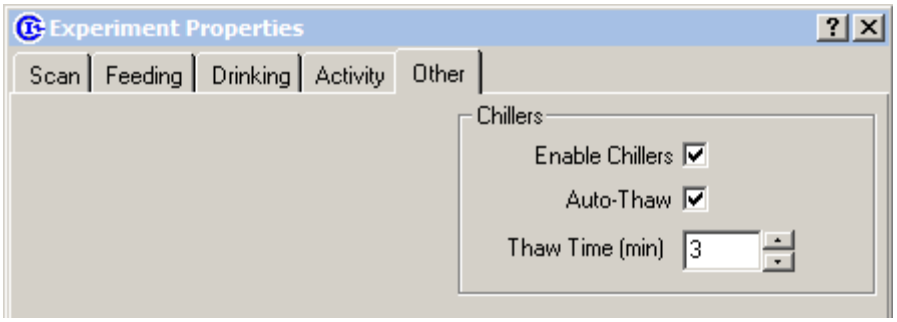


Figure 29 - Urine Chiller Settings

Chapter 4 - Running an Experiment

4.1 Experiment Preparation

The order and timing in the execution of the first steps to running a CLAMS experiment are crucial. Many components have to be powered on in a particular order and allowed to warm-up for a period of time before setting up and running an experiment. These instructions assume that the CLAMS system has been powered down and dormant for at least a day before beginning the next experiment.

1. When CLAMS systems are installed, most components are usually wired such that they are controlled by one or two mains power switches. These switches are usually found on bus strips and surge protectors near the system. **Turn on all bus strips and surge protectors associated with the CLAMS system.** Most of the system's blue and silver cabinets have a light that indicates that the device is powered; check all visible devices for power before proceeding.

> **Note – CLAMS systems with balances**
>
> If the system contains any balances for monitoring food or urine, they initialize into "Standby" mode and need to be turned on manually. If the balance is not turned on manually before starting an experiment, no data will be collected from that balance. To turn them on, **press the "On/Off" button on the front of all system balances.** Also, all balances must have a minimum load on them before being turned on; make sure each balance has its associated feeder or collector on it before turning it on.

2. If it's not already, **make sure the PC used to manage the CLAMS system is powered on and logged in**. If the PC is connected to the internet, it's advisable to disable Microsoft's "Automatic Updates" as these updates can possible restart Windows without user intervention during an active experiment. It's also advised that all energy saving settings be disabled so that the computer will not automatically turn off its hard disk or power-down after long periods without user interaction.
3. **Run Oxymax for Windows and select the appropriate Hardware Configuration**. If the current PC controls a single, fixed-configuration CLAMS system, then select "Yes" when Oxymax asks "Use the current hardware configuration?".
4. After selecting a Hardware Configuration, Oxymax will attempt to initialize the hardware defined therein. If all of the hardware is detected properly, the CLAMS Hardware Status window will automatically minimize itself. If the CLAMS Hardware Status window lists any instruments as having errors, check the specified hardware to make sure that it is powered on and setup properly before clicking "Refresh". **The system cannot warm-up properly if initialization errors are detected.**
5. After the system has been properly initialized, **the gas sensors and controllers require approximately two hours of warm-up time** before the system can be calibrated and used in an experiment.

4.2 Gas Sensor Calibration

In most cases, all of the Oxymax system's gas sensors must be re-calibrated before the start of each experiment. The brief and simple procedure ensures that the readings from the sensors are as accurate as possible before collecting experimental data.

> **Note – Exceptions for calibration**
>
> Calibration is not always necessary before each experiment if the experiments are being run in quick succession. If you're performing several short experiments on the same calendar day, calibrating in between each experiment is not required as long as atmospheric conditions remain stable.

The calibration instructions for the featured gas sensors reflect the proper procedure for currently manufactured systems. Older "legacy" systems may still be supported by the current generation of Oxymax for Windows software with slight differences to procedure. Consult your original Oxymax documentation if the procedures listed in this manual are not viable given your system's setup.

The gas sensors can be calibrated before or after creating a new experiment file. If calibrating before opening an Experiment Configuration, click "Tools >> Calibration" to open the Oxymax Sensor Calibration Window as shown in Figure 30. The same window can be found under "Experiment >> Calibration" if a blank data file has already been created.

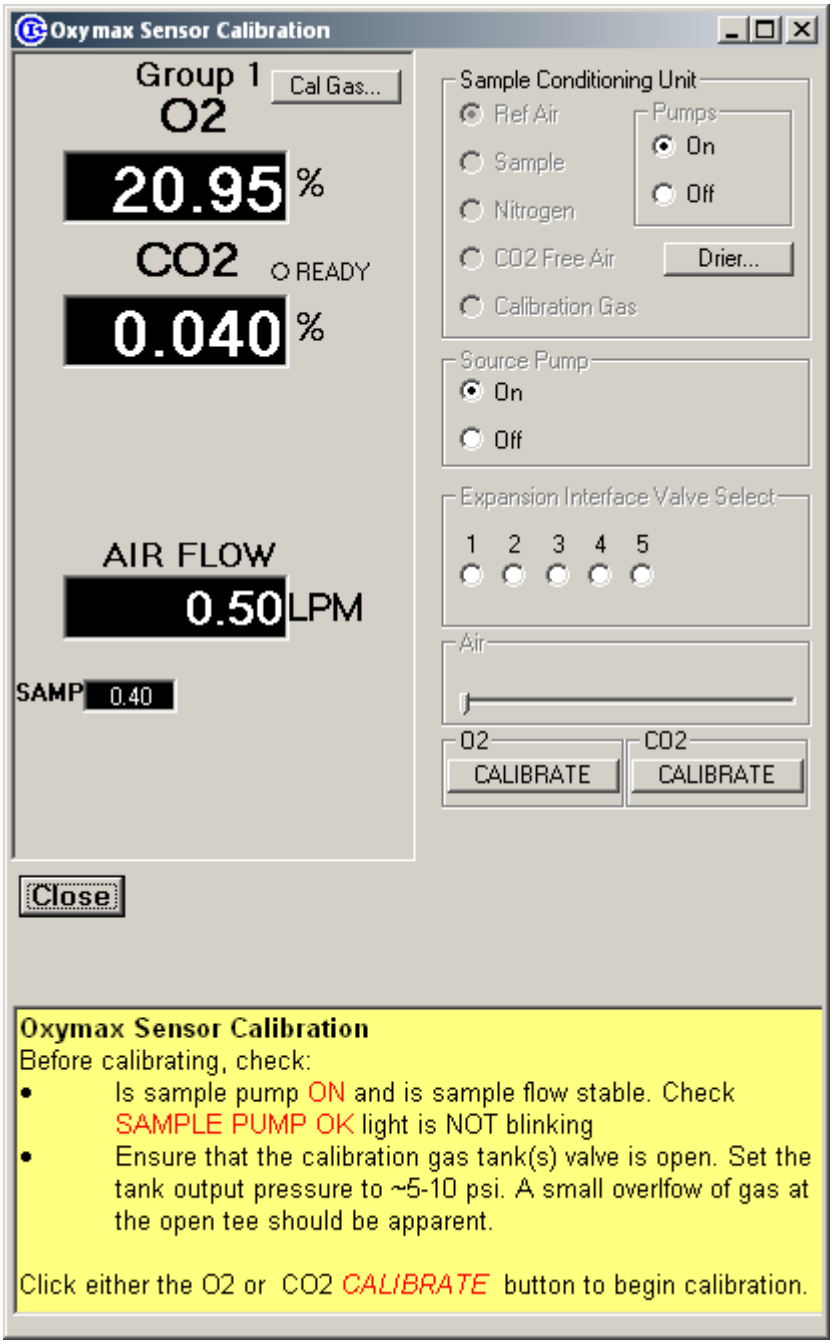


Figure 30 - Calibration Window

Most of the controls on the Oxymax Sensor Calibration Window are for display purposes only and cannot be controlled. Systems with multiple sets of gas sensors will see multiple columns of "Groups" as depicted in Figure 31. The only Calibration Window components that are critical for calibration are the black-and-white gas concentration displays and the "Calibrate" buttons.

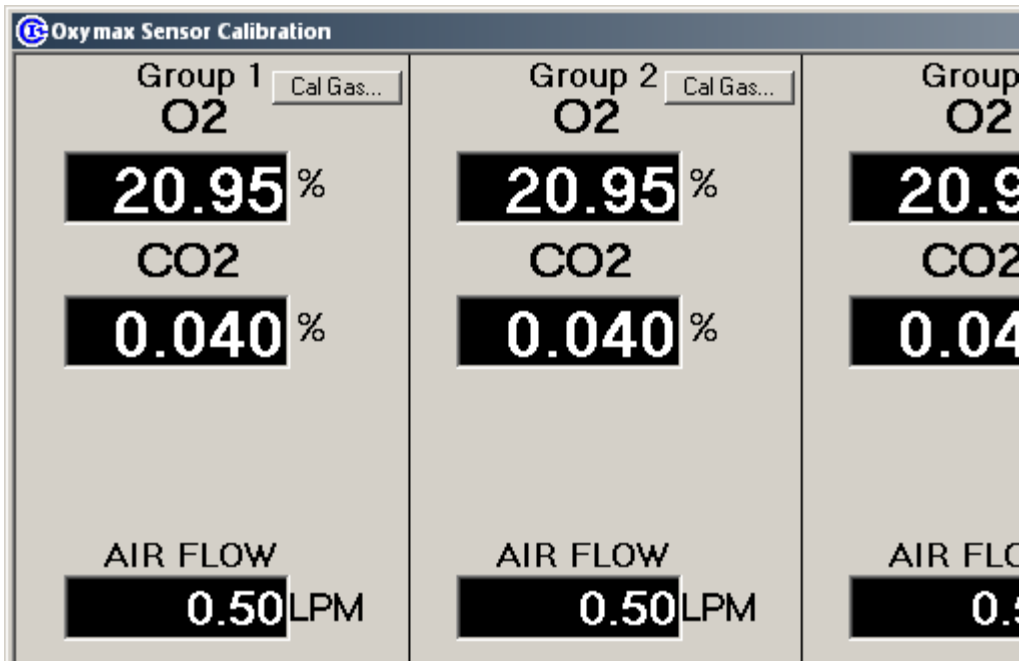


Figure 31 - Calibration with Multiple Sensors

All of the gas sensors use one or more control "calibration gases" to calibrate or validate their measurements. Either automatically or manually, the sensors are tweaked to ensure proper measurement of one or two calibration gases. Usually, the main calibration gas consists of 20.50% Oxygen and 0.50% Carbon Dioxide. Sensors with two-gas calibrations usually also require Nitrogen.

> **Note – Definition of a Calibration Gas**
>
> The purpose of a Calibration Gas is to act as a known control for measurement by a sensor. Calibration Gases are presented with two tolerances: a mixture tolerance and an analysis tolerance. The mixture tolerance implies how precisely the gas was mixed compared to the requested concentration. The analysis tolerance is a measure of how accurate the label on the tank is compared to the actual concentration. Columbus Instruments recommends the use of Calibration Gas that is **mixed to a tolerance of 2% of the requested concentration and analyzed to within 0.02% absolute or 1% of reading** (whichever is smaller). Some gas suppliers refer to these specifications as a "Primary Standard" gas.

Before calibrating, make sure that Oxymax has the proper calibration gas concentrations registered for the gases that will be used during calibration. To set the calibration gas concentrations, click on the "Cal Gas" button next to the sensor group label to display the calibration gas settings frame as in Figure 32. Oxymax assumes the same tanks of calibration gas will be used on all sets of gas sensors so the values do not need to be defined for each group.

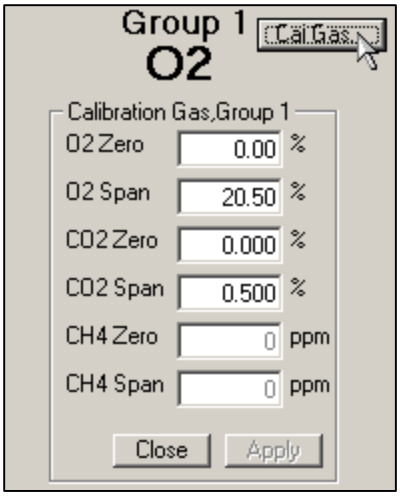


Figure 32 – Standard Calibration Gas Definitions

The Calibration Gas frame shows two concentrations for three different gases: Oxygen, Carbon Dioxide and Methane. Systems without Methane sensors will be unable to edit Methane values. Applications that use Nitrogen for defining a sensor's zero-point will always uses "0.00%" as the concentration of both Oxygen and Carbon Dioxide. The specified concentrations of the calibration ("span") gas should be entered into the appropriate fields as they appear on the Certificate of Analysis that should accompany the calibration gas tank. Click "Apply" to commit the new gas values to memory and then click "Close" to remove the Calibration Gas frame.

Some of the steps in the calibration procedure are common to all sensor types. Before calibrating any sensor, systems that use manual controls to adjust sample flow should be set to about 400 mL/min. The middle of the red indicator ball should float on top of the line that marks the .4 L/min mark as in Figure 33. After adjusting the flow to 400 mL/min, do not re-adjust the flow before starting an experiment. Altering the sample flow requires the system be re-calibrated. Some CLAMS systems have digital flow controllers and do not need to be manually adjusted at all.

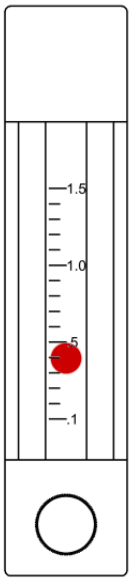


Figure 33 - Sample Flow

When turning on any tank of calibration gas, the outlet pressure as indicated on the tank's pressure gauge should read between 5-10 psi for most systems. Systems with multiple sets of sensors also have multiple sample pumps and will require a higher pressure to ensure a sufficient flow of calibration gas. At the end of the calibration gas tubing is a T-fitting with a small length of tubing on it that's used to exhaust excess gas. When the calibration tank is turned on, a noticeable flow of gas should be apparent from the exhaust tubing. It's always safer to adjust the calibration gas to a pressure that's too high than too low. If the calibration gas pressure is too low, the system can't use pure calibration gas and the sensors will not calibrate properly.

After the system has warmed up for the appropriate length of time, clicking the "CALIBRATE" buttons under the "O2", "CO2" or "OTHER" headers will initiate the appropriate calibration procedure. Exceptions to the aforementioned rules will appear in the following sections regarding the standard gas sensors; if your gas sensor is not listed in this manual, follow Oxymax's on-screen instructions or refer to additionally provided literature to calibrate your sensor.

4.2.1 Fuel Cell Oxygen Sensor

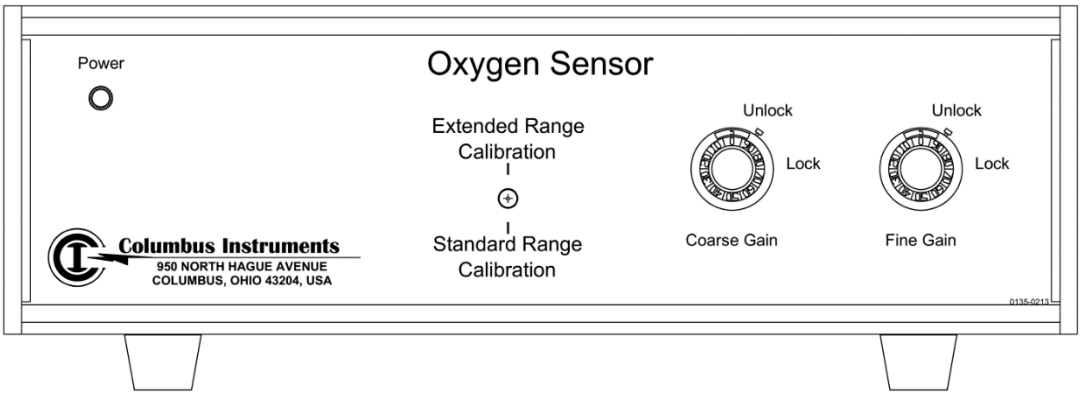


Figure 34 - Original Fuel Cell Oxygen Sensor

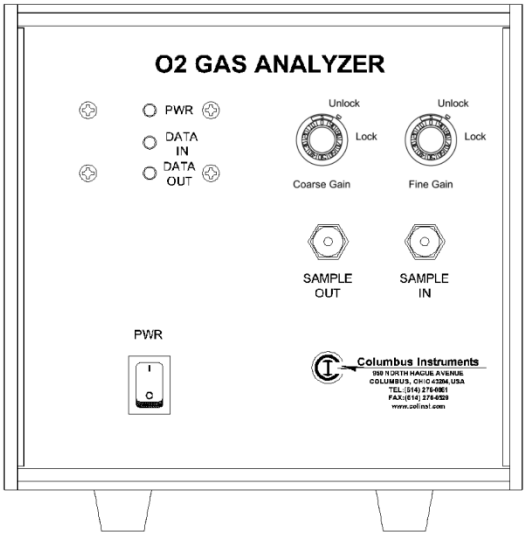


Figure 35 - CI-Bus Fuel Cell Oxygen Sensor

The Columbus Instruments Fuel Cell Oxygen Sensor uses a fuel cell battery to generate small amounts of electricity using Oxygen. The rest of the sensor circuitry determines the Oxygen concentration from the signal the fuel cell generates. Without the presence of Oxygen, the cell doesn't generate electricity and the system interprets that signal as 0.0%. As a result, the Fuel Cell Oxygen Sensor only requires one fixed point to properly calibrate the sensor.

1. **Click the "CALIBRATE" button under the "O2" header** to open the "Oxymax O2 Sensor Calibration" prompt. The prompt contains abridged reminders of the instructions laid out in this manual.
2. If the sample flow(s) for the system is controlled manually on the front of the sample pump, **adjust the sample flow to 400 mL/min**. If you've already adjusted the flow before calibrating another sensor, do not adjust it again.
3. **Turn on the calibration gas** and adjust the pressure so that excess gas is apparent at the exhaust port (~5-10 psi).
4. **Click "NEXT" to start the purging the sensors with the calibration gas.** The progress bar at the bottom of the prompt will indicates the amount of time remaining during the purge period. During this interval, the same lines and gas sensors are being purged of their previous sample and replaced with pure calibration gas.
5. When the purging period is complete, the prompt will display the target Oxygen concentration for the calibration gas. **Use the "Coarse Gain" and "Fine Gain" knobs on each Oxygen sensor to adjust the concentration displayed on the Oxymax Sensor Calibration Window until it matches the target calibration concentration**. The measured concentration will lag adjustments to the knobs by a couple of seconds, so wait for the value to settle before continuing. The displayed value(s) will turn green when the measured value is within 0.01% of the target concentration.
6. After the measured value matches the target concentration, **click "NEXT"** to display the finalization instructions.
7. If all gas sensors have been calibrated, **turn off the flow of calibration gas and click "FINISH"**. Otherwise, just click "FINISH" and proceed immediately to calibrating other sensors.

4.2.2 Paramagnetic Oxygen Sensor

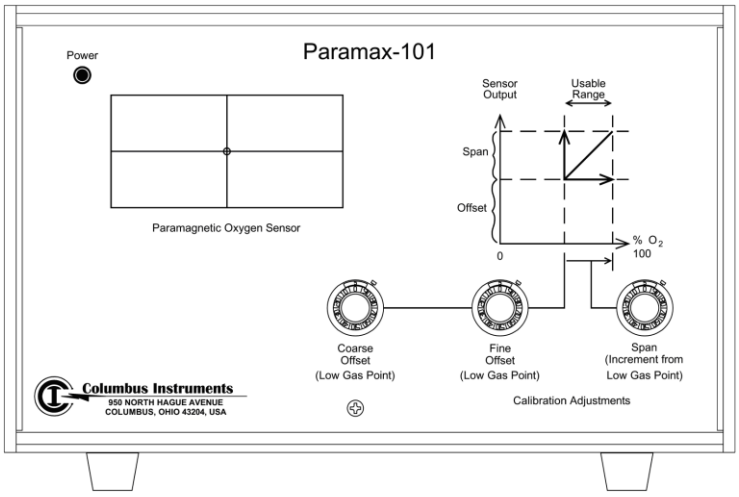


Figure 36 - Original Paramagnetic Oxygen Sensor

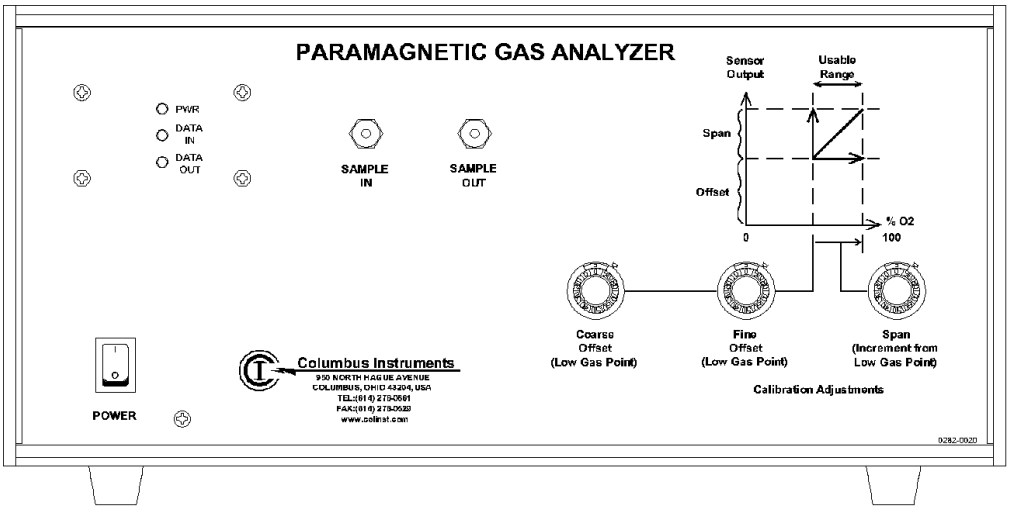


Figure 37 - CI-Bus Paramagnetic Oxygen Sensor

The Columbus Instruments Paramagnetic Oxygen Sensor uses Oxygen's relatively high magnetic susceptibility to measure its concentration in a sample. The sample passes through a magnetic field over a glass dumbbell filled with an inert gas. The magnetically responsive oxygen causes the dumbbell to rotate and the degree of this rotation is used to measure the concentration of Oxygen in the sample. Because of this mechanical implementation, the sensor is very susceptible to motion and it's recommended, when possible, to place this cabinet on a static shelf or to lock the castors on any mobile shelving.

The Paramagnetic Oxygen Sensor requires two points for calibration and therefore requires two different tanks of calibration gas. Usually, pure Nitrogen is used as the "offset" gas and standard calibration gas (20.50% O2) is used for the "span" gas. Depending on the specifications provided at the time of purchase, some systems use a higher concentration gas (~19.00% O2) for the "offset" gas.

> **Note – Nitrogen replaces Soda Lime**
>
> All of Columbus Instruments' Carbon Dioxide Sensors require a two point calibration using a CO2-free gas as a "zero" gas. In most cases, that "zero" gas is created by using Soda Lime to remove Carbon Dioxide from room air. Systems with Paramagnetic Oxygen Sensors usually use the same Nitrogen tank for zeroing both types of sensors.

1. **Click the "CALIBRATE" button under the "O2" header** to open the "Oxymax O2 Sensor Calibration" prompt. The prompt contains abridged reminders of the instructions laid out in this manual.
2. If the sample flow(s) for the system is controlled manually on the front of the sample pump, **adjust the sample flow to 400 mL/min**. If you've already adjusted the flow before calibrating another sensor, do not adjust it again.
3. **Turn on both tanks of calibration gas** and adjust the pressure so that excess gas is apparent at their exhaust ports (~5-10 psi).

4. **Click "NEXT" to start the purging the sensors with "offset" gas.** The progress bar at the bottom of the prompt will indicates the amount of time remaining during the purge period. During this interval, the same lines and gas sensors are being purged of their previous sample and replaced with pure Nitrogen.
5. When the purging period is complete, the prompt will display the target Oxygen concentration for the "offset" gas. **Use the "Coarse Offset" and "Fine Offset" knobs on each Oxygen sensor to adjust the concentration displayed on the Oxymax Sensor Calibration Window until it matches the target calibration concentration (0.00% for Nitrogen)**. The measured concentration will lag adjustments to the knobs by a couple of seconds, so wait for the value to settle before continuing. The displayed value(s) will turn green when the measured value is within 0.01% of the target concentration.
6. After the measured value matches the target concentration, **click "NEXT"** to move to the next step of the calibration.
7. After making sure the "span" gas tank is turned on and providing sufficient flow, **click "NEXT" to start purging the sensors with "span" gas**.
8. When the purging period is complete, the prompt will display the target Oxygen concentration for the "span" gas. **Use the "Span" knob on each Oxygen sensor to adjust the concentration displayed on the Oxymax Sensor Calibration Window until it matches the target calibration concentration**. The measured concentration will lag adjustments to the knob by a couple of seconds, so wait for the value to settle before continuing. The displayed value(s) will turn green when the measured value is within 0.01% of the target concentration.
9. After the measured value matches the target concentration, **click "NEXT"** to display the finalization instructions.
10. If all gas sensors have been calibrated, **turn off the flow of calibration gas and click "FINISH"**. Otherwise, just click "FINISH" and proceed immediately to calibrating other sensors.

4.2.3 Zirconia Oxygen Sensor

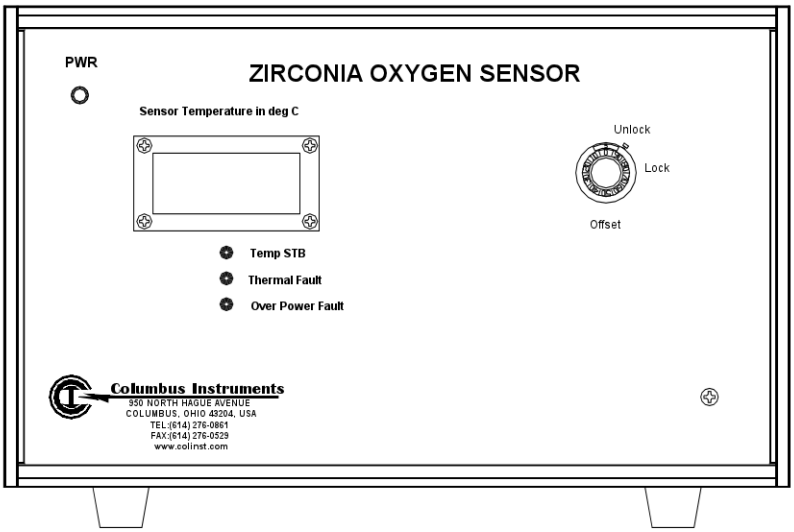
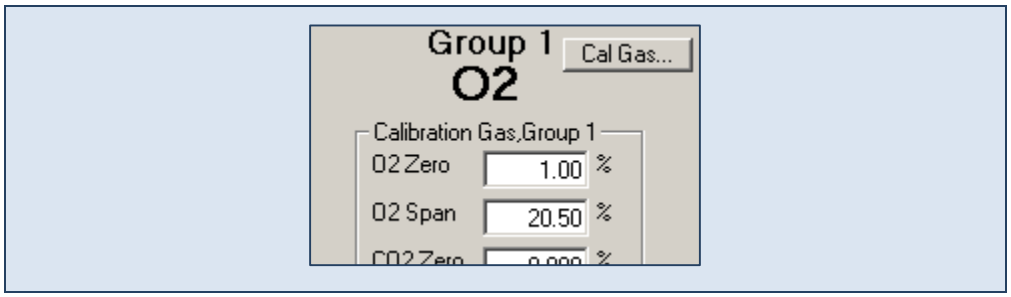


Figure 38 - Zirconia Oxygen Sensor

The Columbus Instruments Zirconia Oxygen Sensor is a differential gas sensor that compares a sample gas to a known, assumedly fixed-concentration, reference gas. The sensor only returns a ratio that represents the concentration of the sample gas to that of the reference gas. The calibration procedure is designed to adjust the sensor such that when the same gas is used for the sample and reference, the ratio returned by the sensor is one. The Zirconia Oxygen Sensor has a small heating element that needs to warm up to about 725 degrees Celsius before calibrating; if the "Temp STB" LED is lit, the sensor is warmed up.

The calibration portion of the procedure does not actually use calibration gas at all. Rather, room air is supplied to both channels of the sensor and then adjusted to return a ratio of one. Then, calibration gas at a known concentration is supplied to the sample channel and the system uses the measured ratio to calculate the concentration of room air. If the calculated value of room air falls into the normal range (20.85%-20.98%), then the sensor is considered calibrated.

> **Note – CalGas settings for Zirconia Sensors**
>
> The Zirconia Oxygen Sensor requires a specific "O2 Zero" parameter in the "CalGas" frame before starting a calibration. Instead of specifying the "zero" gas concentration, you have to specify the target ratio for comparing room air to room air. This ideal ratio is **"1.00"**, and should appear in the "CalGas" settings as depicted here:



1. **Click the "CALIBRATE" button under the "O2" header** to open the "Oxymax O2 Sensor Calibration" prompt. The prompt contains abridged reminders of the instructions laid out in this manual.
2. The Zirconia Oxygen Sensor is very sensitive to changes in sample flow, especially differences in flow across the sample and reference channels. For this reason, both flows are automatically controlled by a pair of external flow controllers (typically a Dual Mass Flow Controller unit) and do not need to be adjusted. **Verify using the display on the Dual Mass Flow Controller that the sample and reference flows read 0.400 LPM**.
3. **Turn on the calibration gas** and adjust the pressure so that excess gas is apparent at the exhaust port (~5-10 psi).
4. **Click "NEXT" to start the purging the sensors with the "offset" gas.** The "offset" gas in this case is actually just room air. The progress bar at the bottom of the prompt will indicates the amount of time remaining during the purge period.
5. When the purging period is complete, the prompt will display the target Oxygen concentration for the calibration gas. **Use the "Offset" knob on each Oxygen sensor to adjust the ratio displayed on the Oxymax Sensor Calibration Window until it reads within 0.0002 of 1.0000**. The measured value will lag adjustments to the knob by a couple of seconds, so wait for the value to settle before continuing. The displayed value(s) will turn green when the measured value is within 0.0002 of the target ratio.
6. After the measured ratio matches the target ratio, **click "NEXT"** to begin purging the sensors with calibration gas.
7. At the conclusion of the purging period, **Oxymax will calculate and display the concentration of room air based on the calibration gas reading**. If the measured concentration falls into the acceptable range for well-mixed room air (20.85%-20.98%), the calibration is considered successful. The measured value for room air will be used as the "O2 In" value for all samples during an experiment (for more on what this means, see **5.1.1 Metabolic Measurements**).
8. **Click "NEXT"** to display the finalization instructions.
9. If all gas sensors have been calibrated, **turn off the flow of calibration gas and click "FINISH"**. Otherwise, just click "FINISH" and proceed immediately to calibrating other sensors.

4.2.4 Automatic Carbon Dioxide Sensor

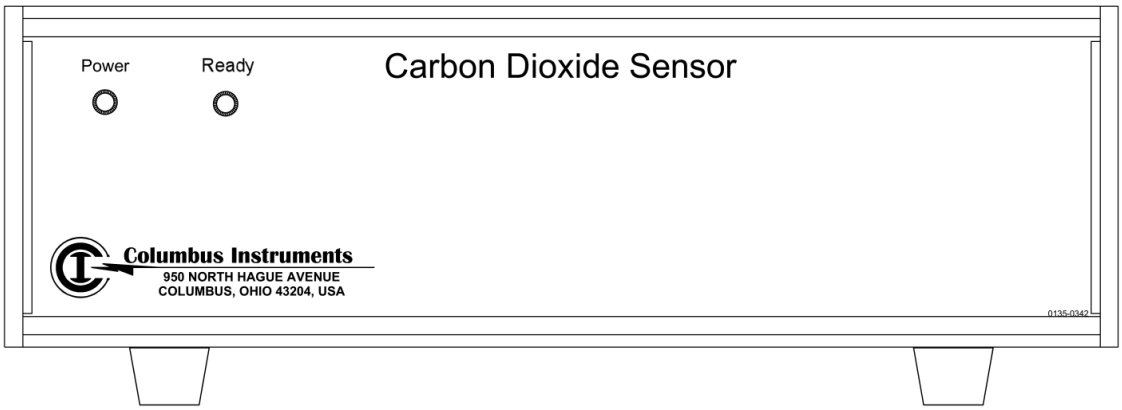


Figure 39 - Original Automatic Carbon Dioxide Sensor

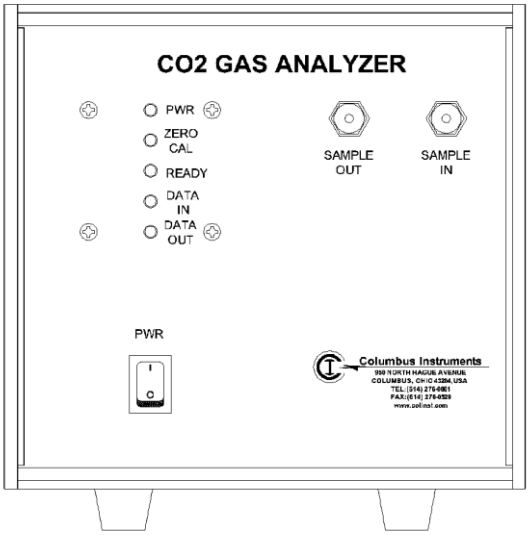


Figure 40 - CI-Bus Automatic Carbon Dioxide Sensor

The Columbus Instruments Automatic Carbon Dioxide Sensor uses a non-dispersive, infrared measurement technique to measure Carbon Dioxide. It has a short warm-up time with a front-mounted "Ready" light that indicates when the sensor is ready to be used.

Once the system is warmed up and the calibration gases are turned on, calibration of the Automatic Carbon Dioxide Sensor is controlled entirely by the software and requires no user-intervention.

> **Note – Ready Light**
>
> The infrared bulb used by the Automatic Carbon Dioxide Sensor is similar to a conventional light-bulb in that it has a filament. After years of use, it's possible for this filament to burn out at which point the sensor will need to be serviced. The unit comes equipped with an LED that indicates if the sensor is warmed up for use. In most cases,

the sensor warms up in about twenty minutes. If, several hours after powering the unit on, the "Ready" indicator light on the front of the unit is not lit, it's possible that the infrared bulb has burned out and requires replacing.

1. **Click the "CALIBRATE" button under the "CO2" header** to open the "Oxymax CO2 Sensor Calibration" prompt. The prompt contains abridged reminders of the instructions laid out in this manual.
2. If the sample flow(s) for the system is controlled manually on the front of the sample pump, **adjust the sample flow to 400 mL/min**. If you've already adjusted the flow before calibrating another sensor, do not adjust it again.
3. **Turn on the calibration gas** and adjust the pressure so that excess gas is apparent at the exhaust port (~5-10 psi). If the system uses Nitrogen for an "offset" gas instead of Soda Lime, turn that tank on as well. Otherwise, check to see that the column of white, granular Soda Lime is secured to requisite tubing.
4. **Click "NEXT" to start the purging the sensors with the "offset" gas.** The progress bar at the bottom of the prompt will indicates the amount of time remaining during the purge period. During this interval, the same lines and gas sensors are being purged of their previous sample and replaced with CO_2-free gas.
5. When the purging period is complete, **the system will measure the "zero" point of the Carbon Dioxide sensor**. During this time, the "Ready" light on the unit will go dark for a few seconds; this is normal.
6. After the "zero" point of the sensor has been established, the system will begin purging the sensors with "span" gas.
7. When the purging period is complete, **the system will measure the gain necessary to calibrate the sensor**.
8. After the gain has been calculated, Oxymax will display a prompt listing the calculated offset and gain values necessary to the Carbon Dioxide Sensor(s). **Turn off the flow of calibration gas(es) and click "FINISH"** to commit this calibration to memory and implement it for the next experiment. If the Oxygen sensor hasn't been calibrated yet, just click "FINISH" and proceed immediately to calibrating it.

4.2.5 Manual Carbon Dioxide Sensor

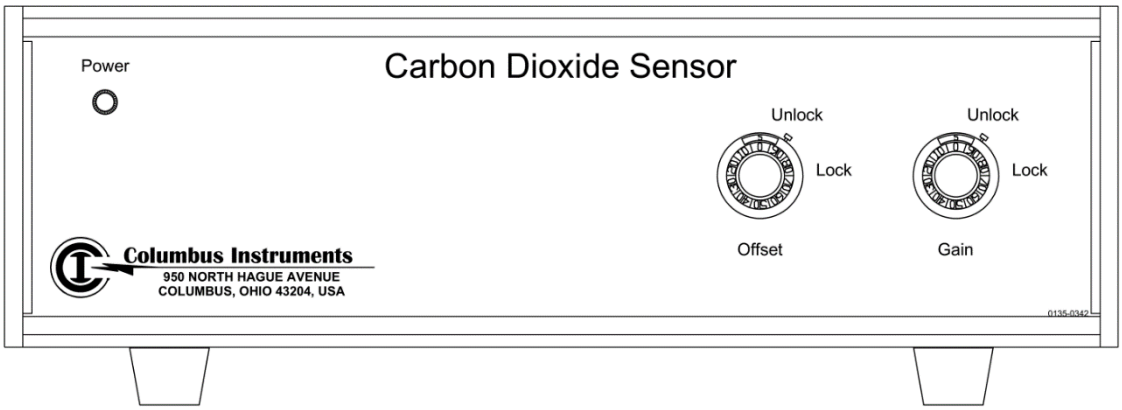


Figure 41 - Manual Carbon Dioxide Sensor

The Manual Carbon Dioxide Sensor is another variation of infrared CO_2 sensor. It calibrates and performs similarly to the Automatic Sensor, but the offset and gain are adjusted by-hand with the software's assistance.

1. **Click the "CALIBRATE" button under the "CO2" header** to open the "Oxymax CO2 Sensor Calibration" prompt. The prompt contains abridged reminders of the instructions laid out in this manual.
2. If the sample flow(s) for the system is controlled manually on the front of the sample pump, **adjust the sample flow to 400 mL/min**. If you've already adjusted the flow before calibrating another sensor, do not adjust it again.
3. **Turn on the calibration gas** and adjust the pressure so that excess gas is apparent at the exhaust port (~5-10 psi). If the system uses Nitrogen for an "offset" gas instead of Soda Lime, turn that tank on as well. Otherwise, check to see that the column of white, granular Soda Lime is secured to requisite tubing.
4. **Click "NEXT" to start the purging the sensors with the "offset" gas.** The progress bar at the bottom of the prompt will indicates the amount of time remaining during the purge period. During this interval, the same lines and gas sensors are being purged of their previous sample and replaced with CO_2-free gas.
5. When the purging period is complete, the prompt will display the target Carbon Dioxide concentration for the "offset" gas. **Use the "Offset" knob on each Carbon Dioxide sensor to adjust the concentration displayed on the Oxymax Sensor Calibration Window until it matches the target concentration**. The measured concentration will lag adjustments to the knobs by a couple of seconds, so wait for the value to settle before continuing. The displayed value(s) will turn green when the measured value is within 0.001% of the target concentration.
6. After the "zero" point of the sensor has been established, **click "NEXT"** to move to the next step of the calibration.
7. As the prompt instructs, make sure the calibration gas is turned on and providing sufficient flow. **Click "NEXT"** to start purging the sensor with the calibration gas.

8. When the purging period is complete, the prompt will display the target Carbon Dioxide concentration for the "span" gas. **Use the "Gain" knob on each Carbon Dioxide sensor to adjust the concentration displayed on the Oxymax Sensor Calibration Window until it matches the target concentration**. The measured concentration will lag adjustments to the knobs by a couple of seconds, so wait for the value to settle before continuing. The displayed value(s) will turn green when the measured value is within 0.001% of the target concentration.
9. After the gain has been set, **click "Next"**.
10. **Turn off the flow of calibration gas(es) and click "FINISH"** to complete the calibration of the Carbon Dioxide Sensor. If the Oxygen sensor hasn't been calibrated yet, just click "FINISH" and proceed immediately to calibrating it.

4.3 Starting an Experiment

After configuring the experiment and properly calibrating the gas sensors the system is ready to start an experiment. Subjects can be loaded into the system chambers at nearly any point during the system setup after Oxymax for Windows has initialized the system.

> **Note – Subject acclimation**
>
> Most research protocols require subjects to be given "acclimation time" before their generated data can be used. This amount of time varies from a couple of hours to an entire day. For users who require only a couple of hours of acclimation time it's suggested that the animals are placed in the CLAMS chambers as soon as the software is initialized and warming up. In this case, when the system is done warming up the subjects will have been acclimated. When a longer acclimation time is required, it's suggested that the experiment be started as soon as the system is warmed up and data recorded during the acclimation period be ignored.

To start the currently configured experiment, select "Experiment >> Run" from Oxymax main menu. If there are any unresolved hardware errors from when Oxymax was first started, a reminder prompt will appear as in Figure 42.

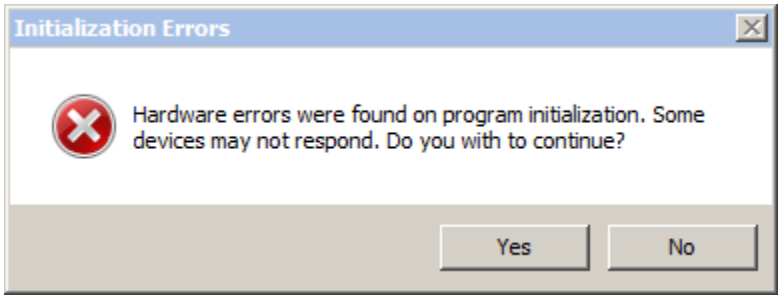

Initialization Errors
Hardware errors were found on program initialization. Some devices may not respond. Do you with to continue?
Yes
No

Figure 42 - Hardware Error Prompt

It's recommended that all hardware errors be resolved before starting an experiment, but the experiment can proceed without the functionality of the failed units.

Oxymax will program any necessary devices with the experiment settings and make a final check that all equipment has been initialized properly. During this time, counters are re-initialized and scales are tared before the experiment. If Oxymax fails to initialize any of these CLAMS devices, a window labeled "Initialization Error Log" detailing device errors will appear along with a prompt as in Figure 43.

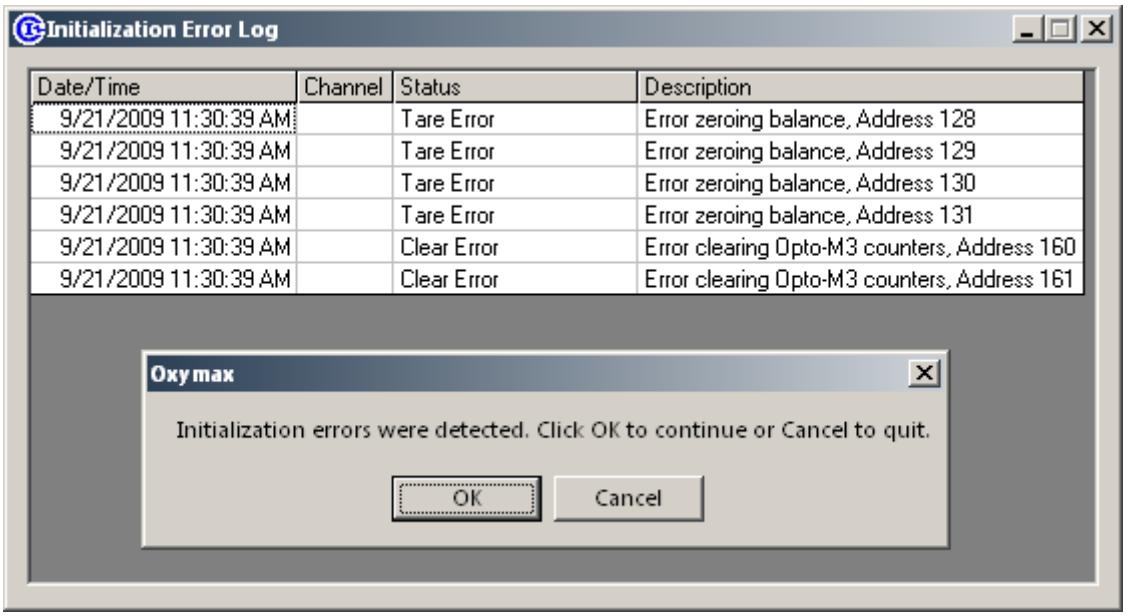


Figure 43 - Initialization Error Log

If all CLAMS hardware is plugged and powered on, none of these error messages should appear when an experiment starts. After initializing all of the CLAMS hardware, Oxymax will begin the measurement cycle and display the status of the current experiment as shown in Figure 44.

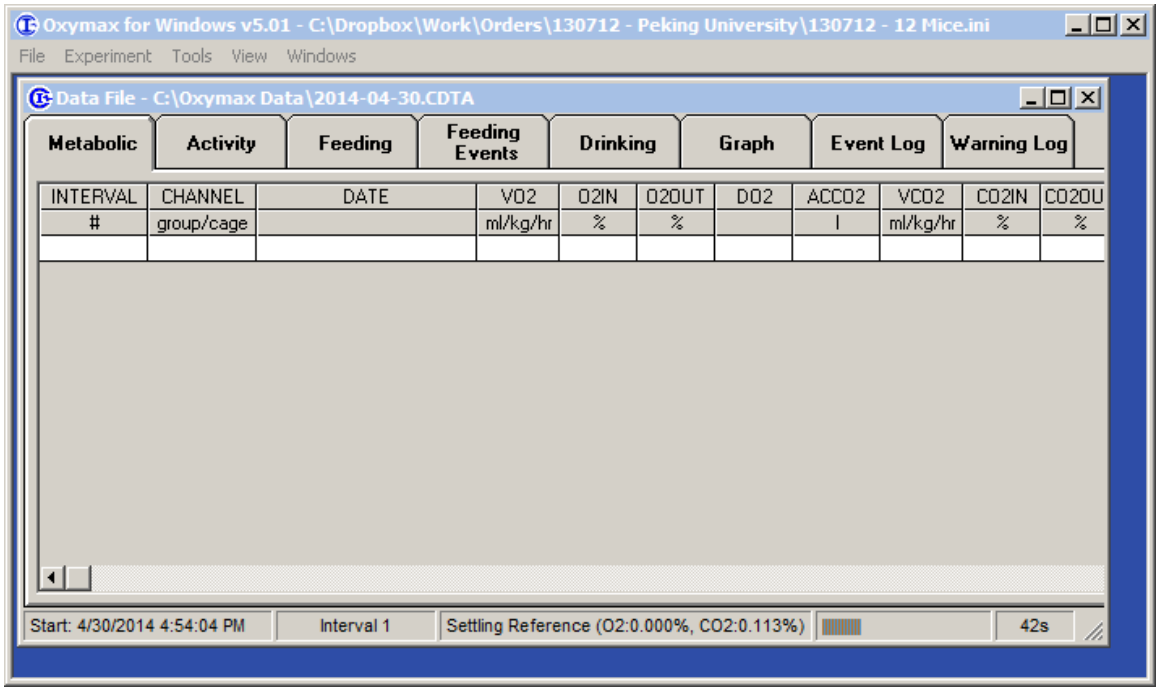


Figure 44 - A Running Experiment

While the experiment is running, the tabs along the top of the data file separating the measurement categories can be used to review all of the currently collected data for this experiment. The bar at the bottom of the data file displays the current status of the running experiment.

- **Start** – The date and time at which the experiment started.
- **Interval** – This is the current interval being measured.
- **"Settling Reference"** – Displays the currently running action for the current measurement. This pane also displays the current concentrations from all of the system's gas sensors.
- **Progress Bar** – Provides a visual representation of the time remaining in the current action.
- **"42s"** – Displays the amount of time remaining in the current action.

While an experiment is running, it may become necessary to open the chambers lids to give the animals various compounds or placebos. The removal of the chamber lid or the addition of any external stimulus can have an effect on the acquired data. Oxymax for Windows can be used to make register events relevant to the experiment that will be included with the exported data. To add an event to the current experiment:

1. Click "Add Event" from the main "Experiment" Oxymax menu to display the "Add Event" prompt as in Figure 45.

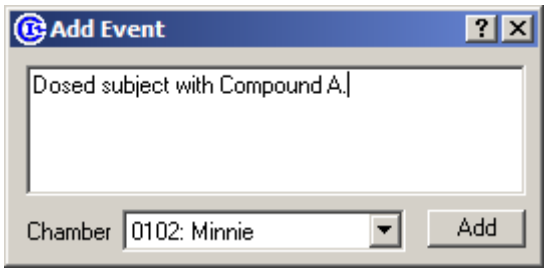


Figure 45 - Add Event Prompt

2. Describe the significant event in the provided text box.
3. Select the chamber for which the event pertains.
4. Click "Add" to commit the event to the data file.

Intervals in which user-generated events have occurred will display an asterisk (*) next to the interval number in the data table. The event will also appear under the "Event Log" tab as in Figure 46.

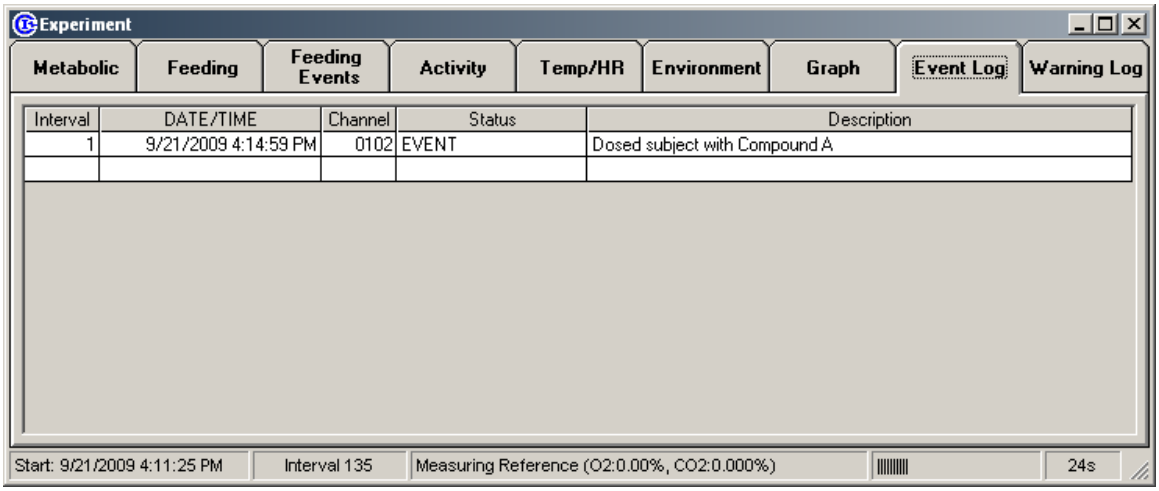


Figure 46 - Event Log

To stop the currently running experiment, click "Experiment >> Stop" from the main menu. When "Stop" is clicked, or any other event occurs that could halt the current experiment, the prompt in Figure 47 will appear to verify the experiments completion.

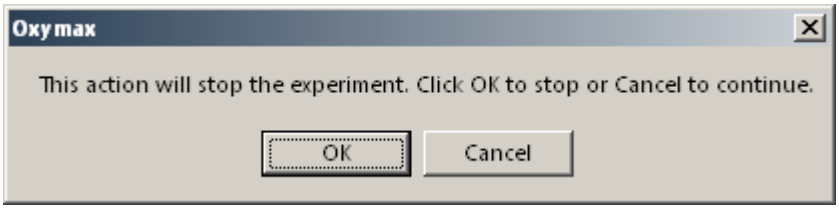


Figure 47 - Experiment Stop Verification

4.4 Refilling Feeders

For systems that have food hardware component, it may become necessary during the course of a long experiment to refill feeders without stopping the experiment. Oxymax for Windows has a pair of utilities for removing feeders from service while they are being refilled depending on the type of feeders in the system.

4.4.1 Balance Feeders

To re-fill one or more balance-based feeders while an experiment is running:

1. Click "Experiment >> Refill Feeders" to open the Balance Utility shown in Figure 48.
    - Note: This utility can also be accessed before an experiment configuration has been loaded under the "Tools > Utilities > Balance Utility" menu option.

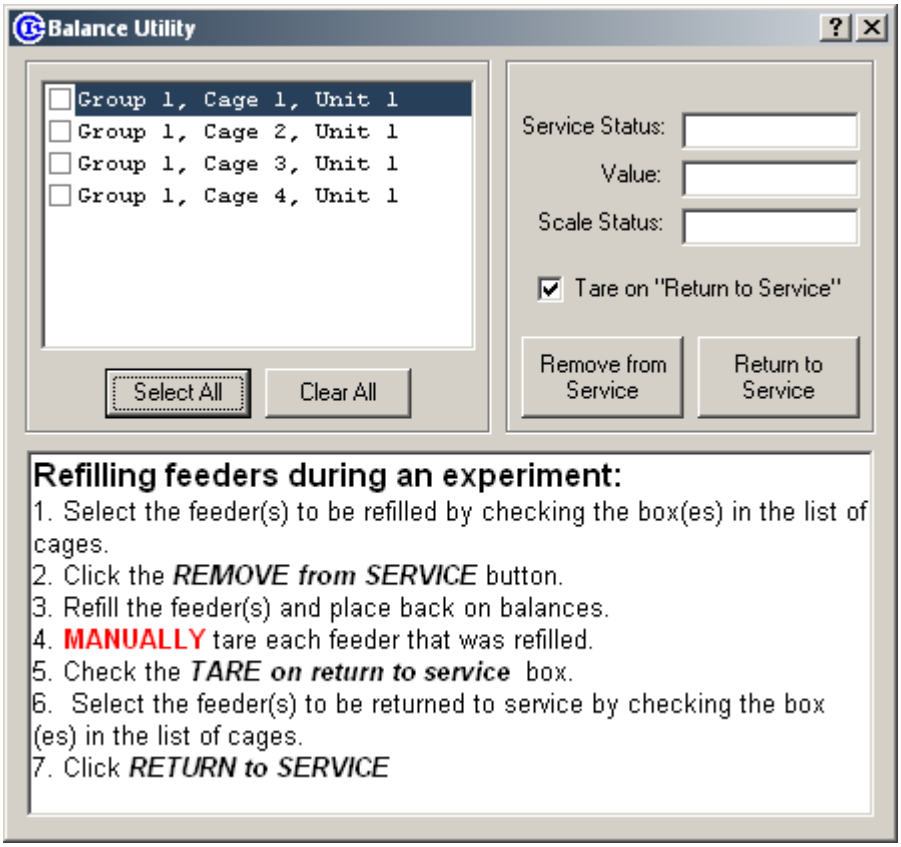

Balance Utility
Group 1, Cage 1, Unit 1
Group 1, Cage 2, Unit 1
Group 1, Cage 3, Unit 1
Group 1, Cage 4, Unit 1
Select All
Clear All
Service Status:
Value:
Scale Status:
Tare on "Return to Service"
Remove from Service
Return to Service
Refilling feeders during an experiment:
1. Select the feeder(s) to be refilled by checking the box(es) in the list of cages.
2. Click the REMOVE from SERVICE button.
3. Refill the feeder(s) and place back on balances.
4. MANUALLY tare each feeder that was refilled.
5. Check the TARE on return to service box.
6. Select the feeder(s) to be returned to service by checking the box (es) in the list of cages.
7. Click RETURN to SERVICE

Figure 48 - Balance Utility

2. Selecting any balance in the list of the left will show its current status on the right.
    - **Service Status** – Indicates whether the balance is currently "In Service" and being measured, or "Out of Service" and being refilled.
    - **Value** – Last stable measurement from the balance.
    - **Scale Status** – Indicates whether the balance is stable, unstable, or experiencing an error.
3. Check the boxes of any balances that need to be refilled in the table on the left-side of the window.

4. Click “Remove from Service” to prevent Oxymax for Windows from sampling those balances while they are being refilled.
5. Remove the chamber and replace the feeder for all of the balances selected in the utility.
6. When all of the selected feeders have been refilled and placed back on the balances, click “Return to Service” to allow the software to record measurements again.

4.4.2 Load Cell Feeders

To re-fill one or more load cell feeders while an experiment is running:

1. Click “Experiment >> Refill Feeders” to open the Load Cell Utility shown in Figure 49.
    - Note: This utility can also be accessed before an experiment configuration has been loaded as pictured below under the “Tools > Utilities > Load Cell Utility” menu option.

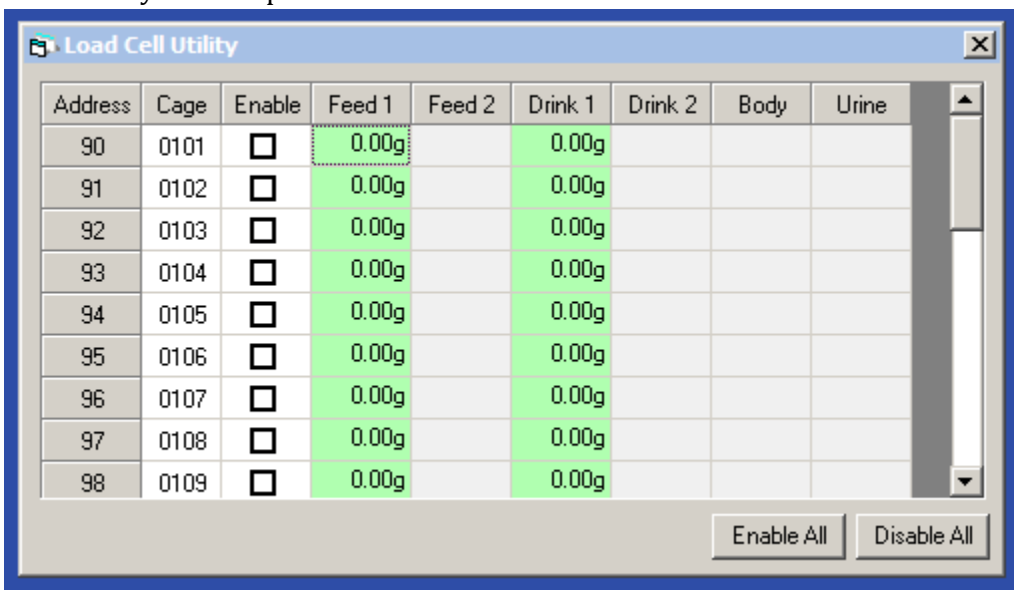


Figure 49 - Load Cell Utility

2. Each of the load cell feeders in the system are displayed in the table at the top of the form.
    - Each cell in the table contains the chamber ID the corresponding load cell belongs to and the current reading from that load cell.
    - Load cells that are not currently enabled will have the word “Disabled” appended to the end of the reading.
    - The background color of the cell indicates the load cell’s current stability. Green cells are stable, red are unstable.
    - Urine collecting cells will also be reset when the controller is re-enabled; it is wise to empty or replace urine receptacles when the feeders are refilled.
3. If the system is also equipped with optional feed control hardware, it might be necessary for the control system to be a particular state while refilling feeders. If

this is the case, select the state the controller should be in while refilling from the "During Refill" dropdown option.

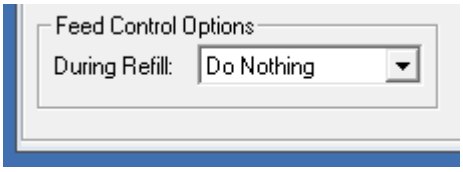


Figure 50 - Feed Control Refill Options

- The controller will return to the state determined by the currently running feed control schedule at the conclusion of the refill.

4. Feeders can only be successfully refilled if taken out of service while they are not being disturbed. Once the subject is away from the feeder being refilled, wait for the corresponding cell in the table to turn green and then uncheck the corresponding "Enable" checkbox in the table. The word "Disabled" will appear next to the corresponding feeder value in the table and, if present, the feed control system will move to the selected state.
    - Given the overhead involved in removing and refilling load cell feeders, it's advisable to refill them completely one at a time. However, the utility does offer the option to "Disable All" and "Enable All" feeders at once should it be necessary to do so.
5. It's at this time the feeder can be removed and refilled. The procedure for removing and replacing the feeder in the chamber will vary by chamber design. Note that depending on the size and design of the feeder it may be necessary to disregard metabolic data collected while the feeder is out of service due to the opening created in the chamber by the feeder's removal.
    - The status of out of service feeders will be logged as part of the data but it is also advised that an event is registered manually for clarity.
6. Once the feeder has be reassembled and reattached to the chamber, wait for the load cell to stabilize and then check the "Enable" box to tare and enable it for service.

4.5 Monitoring Real-Time Position

Position data can be recorded at a high sampling rate on systems with X and Y-Axis activity sensors. Selecting the sampling rate for the compatible activity monitors is explained in **3.3.4 Activity Properties**. The calculated center position of an animal in its chamber can be observed in real-time from the "Activity Monitor" accessible from the "Tools" menu both before and during an experiment. An example of the Activity Monitor is pictured below in Figure 51.

Oxymax for Windows will draw the selected chamber as its defined in the Hardware Configuration file. Before an experiment, the displayed chamber designation is the channel number. While an experiment is running, the chambers can be selected by subject ID. The

currently calculated position is displayed as an index in the corner. See **5.4 Position Analysis** for details on this index.

> **Note – Older configurations**
>
> Position Monitoring has not always existed in Oxymax. If your system has X and Y-axis activity sensors but the chamber displayed on the Activity Monitor doesn't match your chamber, please contact

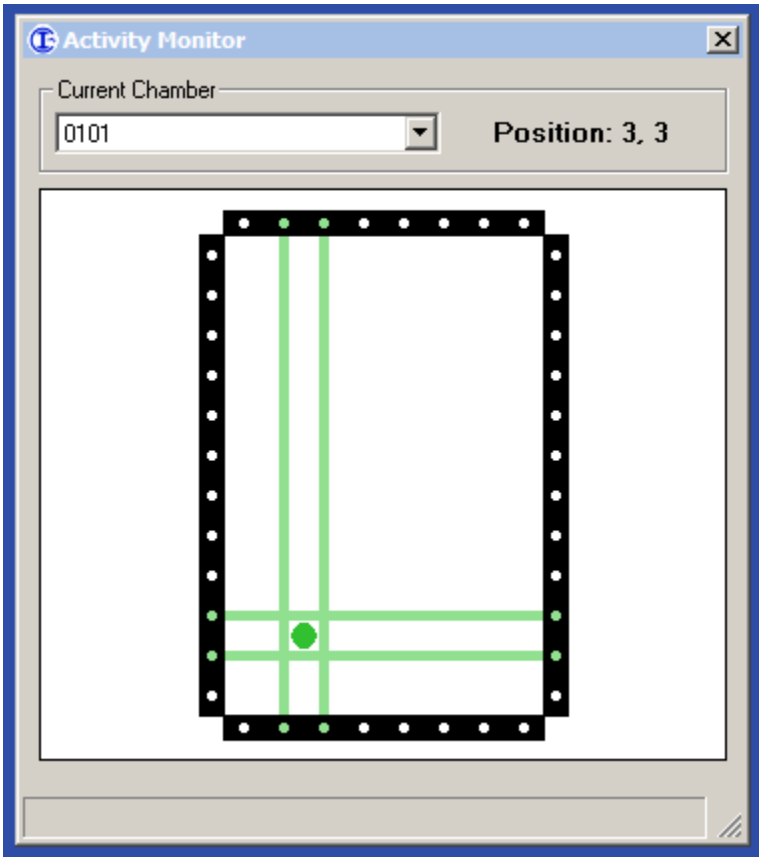


Figure 51 - Activity Monitor

Broken beams are displayed as thin, green lines. Beams broken along the Z-axis – in the upper plane of the chamber – appear as thick green lines. As long as beams are broken on both axes the plot will display the currently calculated center-position of the subject. Use the Activity Monitor before setting up an experiment to make sure that the activity sensors on each chamber are working properly and that there are no outside disruptions breaking beams.

> **Note – Chambers**
>
> Each CLAMS system can be configured with a variety of features that sometimes incompatible with animal position monitoring. Features like Running Wheels have the

potential to block most if not all of the beams on an axis in the chamber. Position monitoring is only available when both X and Y-axis sensors are largely unobstructed.

4.6 Graphing the Current Experiment

Oxymax for Windows comes with a rudimentary graphing utility for quickly observing trends in up to two measurements while an experiment is running. The same utility can be used to generate graphs of data from completed experiments. To create a graph:

1. Click the "Graph" tab at the top of the current data file. Because the graph has not yet been setup, the frame will be empty except for the message, "No Data Available".
2. To select data for display, click the left-most icon in the toolbar above the blank frame. When the mouse cursor rests above the icon, a tool-tip will appear that reads "Select Data". The "Graph Setup" panel will appear as in Figure 52.

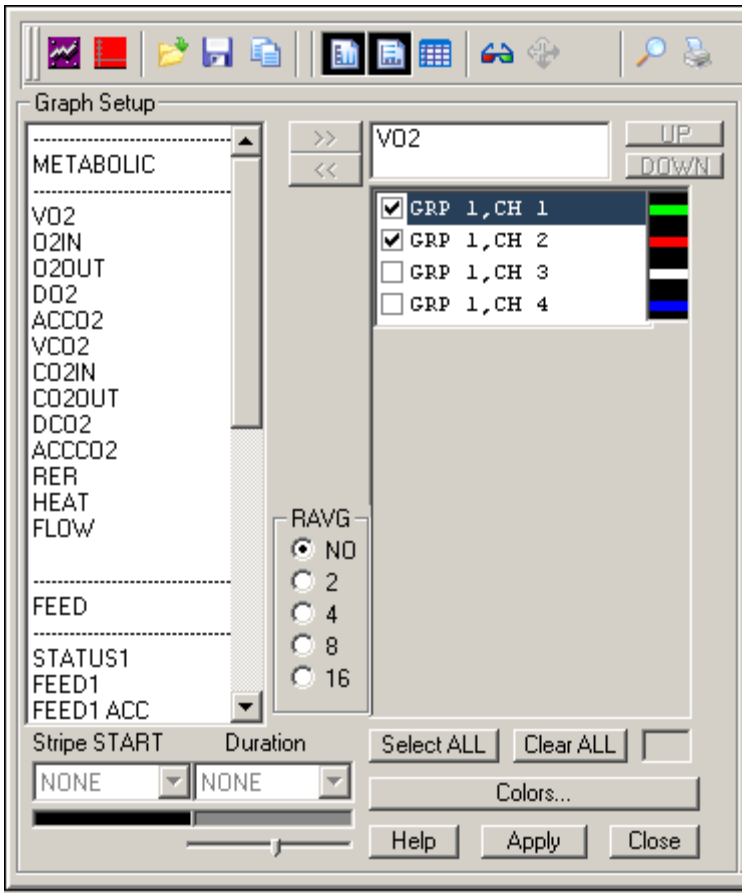


Figure 52 - Graph Setup Panel

3. The available measurements in the current experiment appear in the left table under the same categorical headings as the data file's tabs. Select a measurement in the list and click ">>" to copy it to the list of displayed measurements on the right. The "Up" and "Down" buttons can be used to re-order the displayed measurements.

4. The list on the right contains every chamber in the system. Check the box next to display their data on the graph.
5. Click "Apply" to generate a plot featuring the selected subjects and measurements. Figure 53 shows a plot based on the parameters selected in Figure 52.

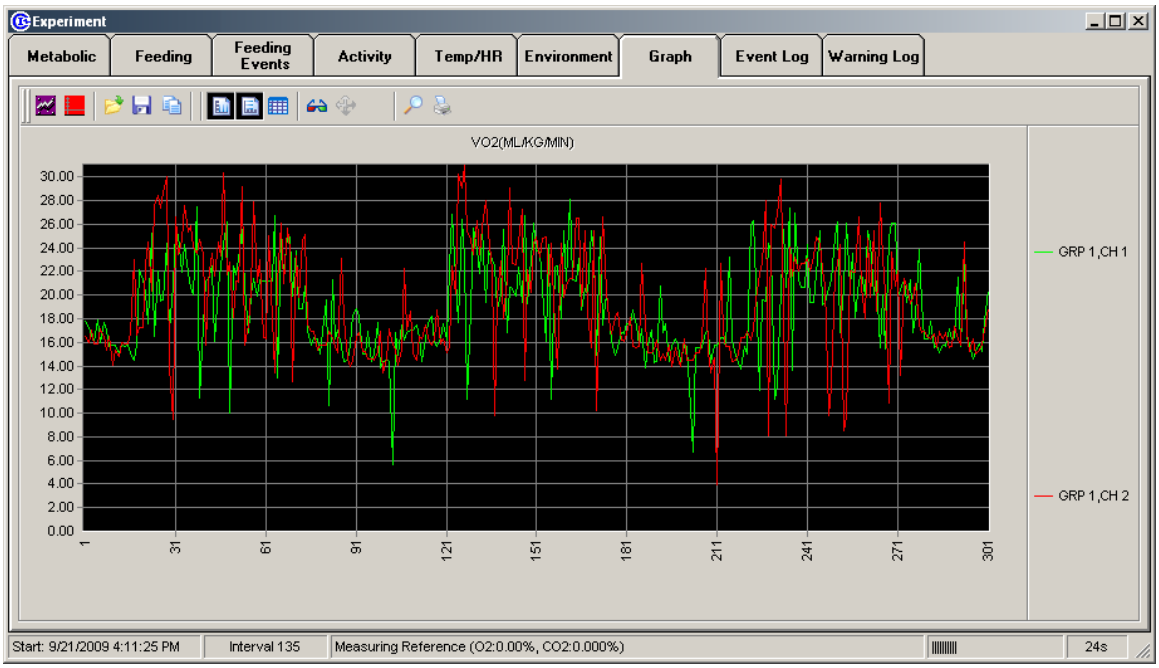


Figure 53 - Example Data Graph

The legend for the graph is on the right-side of the window and lists each of the selected chambers and the color that chamber's data appears in. The colors for both the subjects and the background can be altered to improve readability. On the "Graph Setup" frame, right-clicking any of the colored bars on the subject list displayed the color selection window in Figure 54.

Figure 54 - Color Selection Window

Chapter 5 - Data

5.1 Understanding Data

This section outlines and explains the measurements made by Oxymax and how to interpret the results. Oxymax for Windows stores experiment data in several files that are generated at the start of an experiment. Data is always written to the files as it's acquired so experiment data is preserved in the event of a computer or system power failure. Data files generated by Oxymax for Windows for a particular experiment always have the same filename but different extensions. Files with the same name should always be kept together if moved on the hard drive or to a portable storage device. Each of the file types stores a different kind of CLAMS data; not all users should expect to see every one of the file types described below.

- **.CDTA** – The primary CLAMS data file format that contains all of the synchronous measurements and settings for an experiment. Every possible measurable device associated with a particular chamber is measured at the same its metabolic measurements. Non-metabolic measurements are taken in addition to other periodic or event-driven measurements of the same devices.
- **.BDTA** – Asynchronous feeding event data.
- **.FDTA** –High frequency feeding data.
- **.ADTA** – High frequency activity (infrared beams and running wheels only) data.
- **.PDTA** – Position data.
- **.WDTA** – Animal mass data.
- **.MDTA** – Environmental data.
- **.ELOG** – Warning log information

> **Note – CLAX support**
>
> Depending on the version of CLAX in use, some of the files above may not import for analysis in CLAX.

Most of the tables in the CLAMS data files include interval and time information. Also, the subject to which the measurement pertains is referred to by its chamber identification number under the heading "Channel". "0101" indicates the first chamber in the first group, "0205" indicates the fifth chamber in the second group, etc.

5.1.1 Metabolic Measurements

The crux of CLAMS' metabolic system is the determining of consumption and production rates of Oxygen and Carbon Dioxide. Oxymax makes these measurements by comparing the concentrations of gases being supplied to the chambers to those of the gases leaving the chambers. The difference, or "delta", across the cage is the contribution to the system by the animal in the chamber. The supply gas (room air) depends entirely on the stability of the room in which the system is set up. For this reason, Oxymax makes routine reference measurements of the room air.

Anytime Oxymax switches sample tubes there is an inherent delay between the time a new sample tube is selected and the time the gas from that tube completely flushes the previous sample out of the sensors. This time differs based on the flow rate, length of the tube and the response time of the sensor. This length of time is referred to as a "settle time". During this settle time, Oxymax for Windows queries the gas sensors for their concentrations but does not use the results to calculate the measurement for that sample. The interval directly following the settle time is referred to as the "measurement time". During this time, the sensors are queried once a second and the average reading over the measurement time is recorded as the measurement value.

Oxymax for Windows repeatedly takes reference measurements after a specified number of chamber measurements. Figure 55 shows the first four cages of an example experiment with both settle times set to 90 seconds and both measure times set to 30 seconds.

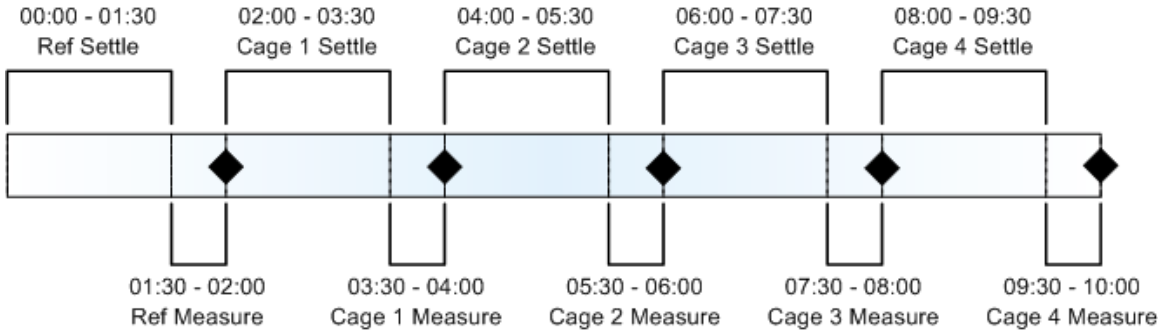


Figure 55 - Example Experiment Timeline

If the example system had only four chambers and was set to make a reference reading every four chambers, the timeline in Figure 55 would repeat until the experiment was stopped. The referencing frequency (in samples) is usually calculated such that a reference measurement takes place at least every 20-30 minutes. Research environments with especially stable ambient conditions can afford to go longer periods in between references while noisy environments should reference more frequently. It is also recommended that the number of samples in between references be evenly divisible by the number of *enabled* cages in the system. For example, a sixteen chamber system with twelve enabled chambers could reference every three, four, six or twelve samples. It's not impossible to use a referencing frequency that does not divide evenly into the number of enabled cages, but as Figure 56 shows, the result of doing so is that chamber sample intervals will vary in length.

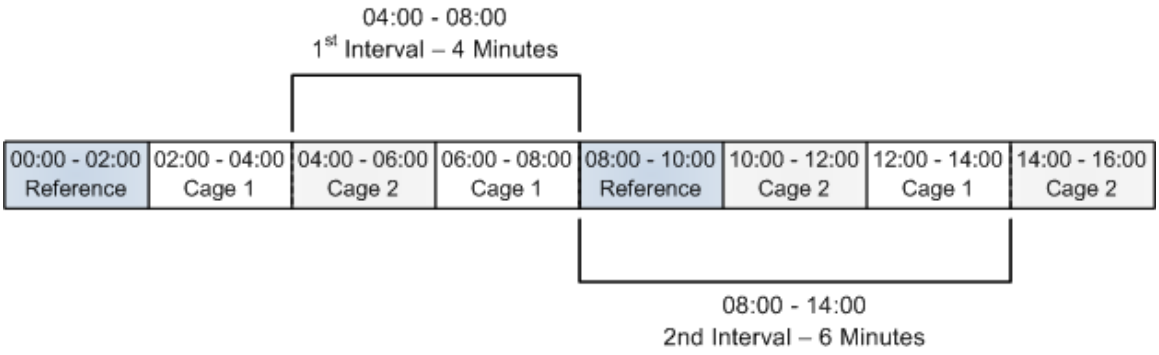


Figure 56 - Unequal Sample Intervals

Figure 56 shows an experiment with two enabled chambers set to reference every three samples. In this case, the sample interval for any either chamber alternates between four and six minutes.

For systems that contain only one set (Oxygen and Carbon Dioxide) of gas sensors, only one chamber can be sampled at a time. But, systems with multiple sets of sensors can sample one chamber for every set of sensors. Chambers in multi-sensor systems are placed into "groups" based on the pair of sensors that samples them. Chamber identification numbers are prefixed by the group number; the fifth chamber of the third group would be referred to as "0305". Every chamber with the same index in every group is sampled at the same time; for example, 0101, 0201, 0301, etc would be sampled simultaneously. If one chamber in a particular group, 0204 for example, were disabled for an experiment, the sensors for "Group 2" would not record data while chambers 0104 and 0304 were being sampled. If all the 04's in each group were all disabled, then Oxymax would skip channel four and proceed to the next channel in which at least one group had an enabled chamber. For this reason, the most efficient way to organize a small quantity of subjects across a multi-group system would be to spread them evenly over the lowest numbered channels (0101, 0201, 0301, 0102, 0202, 0302, etc).

All of the metabolic measurements are presented by Oxymax for Windows in shorthand form. The following list details all of the measurements taken by Oxymax. The equations behind the calculations of derived measurements are presented in **5.4 Position Analysis**.

- **Gas Measurements** – Oxymax for Windows records the same type of measurements for each gas Oxymax monitors. For simplicity's sake, rates for gases that are consumed (Oxygen) and those that are produced (Carbon Dioxide, Methane, etc) are both presented as positive measurements. In the explanation of measurements below, "x" takes the place of "O2" (Oxygen), "CO2" (Carbon Dioxide) or "CH4" (Methane).
  - **Vx** – The rate of gas consumption/production normalized to animal body mass. The units for this measurement are selectable, but are typically displayed in mL/kg/hr.

- **xIN** – The measured concentration of room air taken at the last reference. The generally excepted values for room air concentrations are:
  - **Oxygen** – 20.88%-20.96%: Readings greater than 20.97% are usually the result of an insufficiently long warm-up period or, in the case of the Fuel Cell Sensor, a significant increase in ambient temperature since calibration.
  - **Carbon Dioxide** – 0.040%-0.110%: This concentration depends highly on the air quality in the region in which the system is installed. It also depends on the turnover rate for the ventilation system and the volume of the room compared to other CO_2 contributors like people. Values slightly below 0.040% are acceptable, but no environment should read 0.000% unless a chemical CO_2 scrubber is being used.
- **xOUT** – The measurement of chamber air taken for the sample. Chamber concentrations will vary depending on room air concentration, subject contributions and fresh air flow.
- **Dx** – The difference (delta) in concentration between the xOUT and xIN values. Controlling the fresh air flow to the chambers can be used to move these values into the optimal ranges for the sensors. If the difference between the input and output concentrations is too small, it's difficult to measure with any degree of certainty. If the difference is too high, the air will be too "thin" for the subject to breathe regularly and will have an impact on its respiration.
- **ACCx** – The total volume (in liters or milliliters) of gas consumed or produced by the subject.

- **RER** – "Respiratory Exchange Ratio". The unit-less ratio of the rate of Carbon Dioxide production to the rate of Oxygen consumption. When the subject is metabolizing pure fat for energy its RER is close to 0.70. When the subject is metabolizing pure carbohydrates for energy its RER is close to 1.0. The subject's RER can exceed 1.0 during intense exercise or while synthesizing fat from carbohydrates.
- **Heat** – The measure of energy expenditure for a subject. This is presented either as a whole-subject or mass-normalized rate, either in kilocalories or kilojoules.
- **Flow** – The rate of fresh air delivery to the chamber in liters per minute (LPM). The target fresh air flow rate is based mostly on subject mass, but typical values are:
  - **Mice** – 0.50-0.60 LPM
  - **Rats** – 2.0-3.5 LPM
- **Pressure** – This is the pressure in the sample loop as reported by some models of gas sampler. Not all products contain or require the requisite components to record this value. It is not used in the computation of metabolic figures but indicates the state of the sampler during the experiment.

5.1.2 Feed Measurements

Oxymax for Windows makes feeding measurements in several methods to suit a variety of research needs. Feeding data is recorded in three different methods:

- Synchronously with the metabolic data. Consumption data for a chamber is measured and logged at the same time as the metabolic data for that chamber. This data appears under the "Feeding" tab and is stored in the .CDTA file.
- Asynchronously as the animal interacts with its food. Each time the balance is disturbed the software keeps track of how long the animal disturbs the feeder and how much food it removes. This data appears under the "Feeding Events" tab and is stored in the .BDTA file.
- Periodically at a higher rate than the metabolic sampling frequency. This rate is adjustable in the experiment settings. This data is only viewable when it has been exported and is stored in the .FDTA file.

Both the synchronous and high-frequency measurements consist of the same basic measurements:

- **Date** – The date and time the measurement was taken.
- **Status** – If the balance is being disturbed at the time of the measurement it cannot be read and Oxymax for Windows flags the measurement as "Unstable". The software will then record the difference between the last measurement and the last "Stable" reading from the balance. If the balance is "Stable", the value can be read and recorded immediately.
- **Feed** – The amount of food that has been consumed since the last measurement.
- **Feed Acc** – The total amount of food that has been consumed since the beginning of the experiment.

The "Feeding Events" measurements contain additional information about the subjects feeding behavior:

- **Count** – The number of feeding events a balance has recorded.
- **Interval** – The metabolic sampling interval in which the event took place.
- **Date** – The date of the feeding event.
- **Start** – The time at which the feeding event started.
- **End** – The time at which the feeding event ended.
- **Duration** – The length of time the event lasted.
- **Weight** – The amount of food consumed during the feeding event.
- **Accum** – The total amount of food consumed during the experiment.

5.1.3 Drinking Measurements

CLAMS systems offer three different methods for recording liquid consumption: lick counters, Volumetric Drinking Monitors and Load Cell Water Bottles. Lick counters are simple electrical devices that register each time the subject drinks from a sipper tube. The Volumetric Drinking Monitor detects when the subject licks a specially designed sipper tube and delivers a measured volume of liquid to the subject. Load Cell drinking monitors the amount of consumed water by mass rather than volume.

Systems with lick counter devices register the following measurements for each sipper:

- **Licks** – The number of licks that have occurred in the interval.
- **Licks Acc** – The total number of licks that have occurred since the beginning of the experiment.

Systems with Volumetric Drinking Monitors or Load Cell Water Bottles register the following measurements for each sipper (units vary by method):

- **Drink** – The volume/mass of liquid the subject consumed in the interval in milliliters/grams.
- **Drink Acc** – The total volume/mass of liquid the subject consumed since the beginning of the experiment.

5.1.4 Activity Measurements

CLAMS systems can record two different types of activity: locomotion via an infrared beam system and exercise via either free-running wheels or treadmills.

Infrared beams can monitor any or all of the chambers' axes. When using infrared beams data is presented in terms of the number of "beam breaks". For the locomotion axes (X and Y), results are quantified in two different ways: total and ambulatory. For the rearing axis (Z), only total counts are recorded. The differences between these two metrics are:

- **Total** – The total number of times infrared beams were broken during an interval.
- **Ambulatory** – The number of times different beams were broken during an interval. If a subject stands still and begins a repetitive act, such as grooming, it will repeatedly break the same beam. The ambulatory count is only incremented when the currently broken beam is different than the last broken beam.

Running wheels are equipped with a sensor to detect rotation in either direction.

- **Wheel** – The number of complete rotations by the running wheel in an interval.
- **Wheel Acc** – The total number of complete rotations by the running wheel since the beginning of the experiment.

Systems equipped with current-generation (circa 2010) Treadmill Controller units have the option of recording treadmill speed and odometer values during an experiment. The Treadmill Shock Detection unit can be added to record shocks and visits to the shock grid as well. The data stored for these products are:

- **Treadmill Speed** – The current speed of the Treadmill Controller at the time of the sample in meters per minute.
- **Treadmill Odometer**– The current odometer reading from the Treadmill Controller unit. The odometer is reset to zero at the start of the Oxymax experiment. In cases where there is a Treadmill Shock Detection unit in play, this value is recorded from that unit instead which will pause the odometer while the animal is being shocked so it doesn't count towards the distance travelled.

- **Shocks** – For systems that include the Treadmill Shock Detection, this is the cumulative number of shocks that have occurred since the beginning of the experiment.
- **Shock Visits** – For systems that include the Treadmill Shock Detection, this is the cumulative number of visits to the shock grid that have occurred since the beginning of the experiment.

5.1.5 Urine Measurements

Oxymax for Windows monitors urine production using balances or load cells also used to measure food consumption. Urine production data is stored in the same way as the synchronous feeding data is:

- **Urine** – The mass of urine produced by the subject in the given interval in grams.
- **Urine Acc** – The total mass of urine produced by the subject since the beginning of the experiment.

5.1.6 Telemetry Measurements

The telemetry component of CLAMS monitors a subject's core temperature and heart-rate. Columbus Instruments offers two varieties of implantable transmitters that communicate to Oxymax for Windows via a custom antenna affixed to the chamber. One model of transmitter monitors subject core temperature and heart rate, another just core temperature. The following telemetry measurements are recorded on the "Temp" or "Temp/HR" tabs of the data file:

- **Body Temp** – The subject's core temperature at the time of the sample.
- **Heart Rate** – The subject's heart rate at the time of the sample in beats per minute.

In addition to the subject's core temperature, CLAMS can monitor the ambient temperature of the chamber using Columbus Instruments' Iso-Thermex.

- **Cage Temp** – The ambient temperature of the chamber at the time of the sample.

5.1.7 Environmental Measurements

In addition to monitoring subject behavior and health, CLAMS systems with the Columbus Instruments Environmental Monitor can record conditions in the room into the MDTA file. The Environmental Monitor data records the following information:

- **RH Samp** – The relative humidity of the sample gas before drying.
- **RH Purge** – The relative humidity of the dry air used to remove moisture from the sample.
- **RH Amb** – The relative humidity of the environment.
- **Temp Amb** – The ambient temperature
- **Baro Press** – The barometric pressure of the room in millimeters of Mercury.
- **Room Light** – Whether or not the lights were on in the room at the time of the sample.

Contained in the same data file as the Environmental Monitor data is data from the Environmental Controller product if the system has one.

- **Enclosure Temp** – The measured temperature inside the Environmental Enclosure in degrees Celsius.
- **Enclosure Setpoint** – The set-point temperature of the Environmental Controller.
- **LED Hue** – The hue of the LED controls at that time on the standard Hue scale (value between 0 and 255).
- **LED Saturation** – Saturation level of the LED color expressed as a percentage.
- **LED Lightness** – Measure of LED intensity expressed as a percentage. This is a measure of delivered power to the LED's which may not scale evenly compared to actual brightness.

5.2 Exporting Data

By default, Oxymax for Windows stores experiment data in a proprietary format that cannot be read by third-party software. In order to investigate data in other applications, the data must be exported into a format that is compatible with other spreadsheet software (Microsoft Excel, OpenOffice, Apple Numbers, Google Sheets, etc). All data recorded by Oxymax for Windows can be exported as Comma Separated Values (CSV) file which can be read by all major spreadsheet programs.

Oxymax for Windows can make CSV's based on either subject or measurement parameter. Subject-based CSV's contain every measurement recorded for a given subject; parameter-based CSV's contain every subject's data for a given parameter.

To automatically generate all of an experiment's subject-based CSV's:

1. Open up the desired experiment data file.
    a. The data generated from a just-finished experiment is still open after stopping it.
    b. To open a previously saved experiment, instead of clicking "File >> Open Experiment Configuration" after loading a hardware configuration, click "File >> Open Experiment Data" and select the desired .CDTA file.
2. Click "File >> Generate Subject CSV's" to automatically generate CSV files for every subject in the current data file.
3. Each subject may generate multiple files depending on the enabled features of the CLAMS system:
    a. `[Experiment Name].[Chamber ID].CSV` – Contains all synchronous CDTA measurements for the specified chamber.
    b. `[Experiment Name].B[Chamber ID].CSV` – Contains all asynchronous feeding data from the .BDTA file for the specified chamber.
    c. `[Experiment Name].F[Chamber ID].CSV` – Contains all high-frequency feeding data from the .FDTA file for the specified chamber.

d. `[Experiment Name].A[Chamber ID].CSV` – Contains all high-frequency activity data from the .ADTA file for the specified chamber.

To generate CSV's for specific synchronous measurements:

1. Open up the desired experiment data file.
   a. The data generated from a just-finished experiment is still open after stopping it.
   b. To open a previously saved experiment, instead of clicking "File >> Open Experiment Configuration" after loading a hardware configuration, click "File >> Open Experiment Data" and select the desired .CDTA file.
2. Click "File >> Parameters" to open the Parameter File Utility shown in Figure 57. Instructions for generating parameter CSV files are also displayed in the "Instructions" frame of this window.

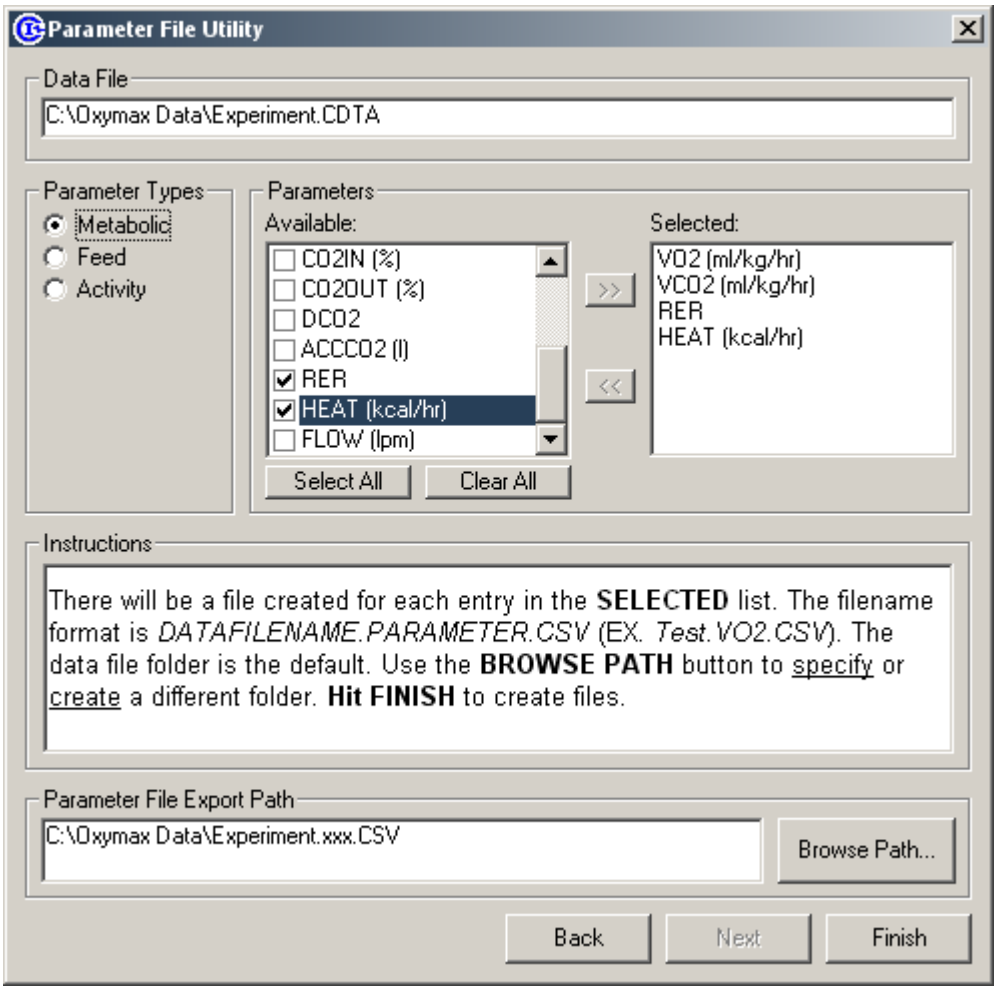


Figure 57 - Parameter File Utility

3. Use the "Parameter Types" list to view the different categories of measurements.

4. Check the box in the "Available" list next to the desired measurements and click ">>" to move them to the "Selected" list.
5. Click "Next" to advance the on-screen instructions.
6. The utility will automatically select the directory that contains the CDTA file as the directory in which to create the generated CSV's. To select another directory, click "Browse Path" and select a different one.
7. Click "Finish" to generate the parameter CSV files.

The filename format for parameter CSV's is: `[Experiment Name].[Parameter].CSV`

5.3 Sleep Analysis

CLAMS systems equipped with infrared activity monitors and running wheels can be used to analyze sleep behavior based on locomotion. For sleep analysis, the high-frequency activity sampling interval is referred to as an "epoch". Sampling intervals of once every ten seconds are recommended for utilizable data. The subject is understood to be sleeping if it fails to break a particular number beams or turn the running wheel a particular number of revolutions over the course of several epochs. The sleeping bout continues until the animal breaks that activity threshold. Research has suggested that four consecutive ten-second epochs with a threshold of zero activity counts is a sufficient indicator of sleep.

> **Note – Sleep Experiment Setup**
>
> Before beginning an experiment in which sleep is an area of interest, be sure to enable high-frequency activity monitoring on the Experiment Properties Window. After loading an experiment template, click "Experiment >> Properties" and select the "Activity" tab. Set the "Activity Sampling Rate" to "10 sec" for the best sleep analysis results.

Sleeping statistics are generated from high-frequency activity data by the Sleep Analysis Utility. To access the utility shown in Figure 58, select "File >> Export >> Sleep Analysis" when Oxymax for Windows has an open data file.

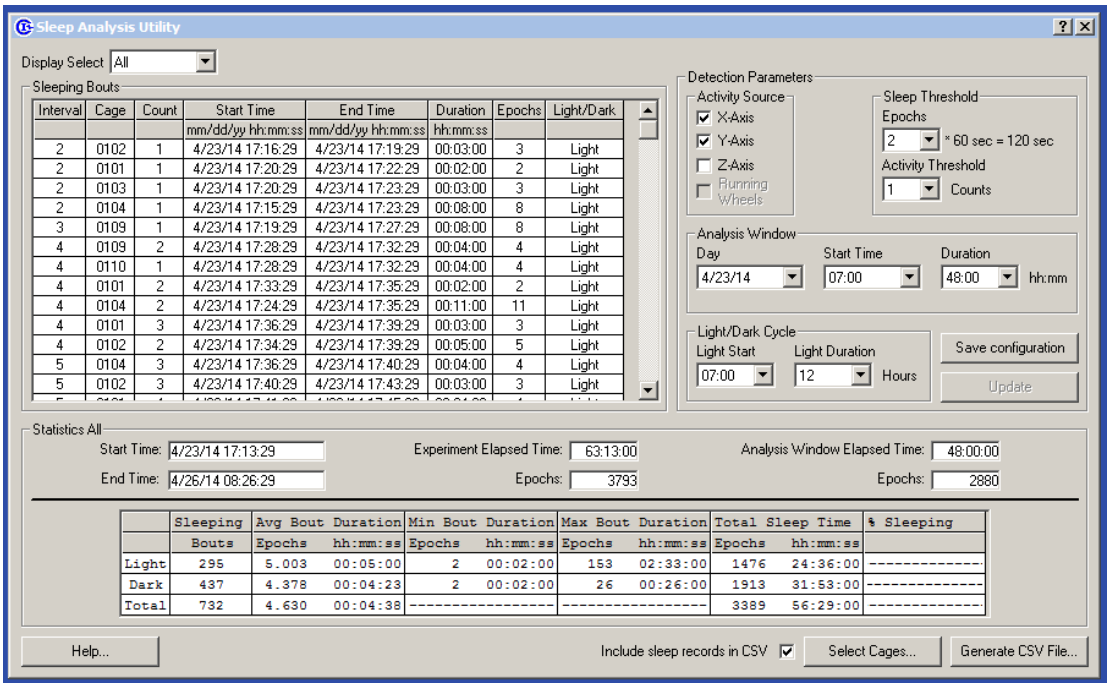

Sleep Analysis Utility
Display Select All
Sleeping Bouts
Interval Cage Count Start Time End Time Duration Epochs Light/Dark
mm/dd/yy hh:mm:ss mm/dd/yy hh:mm:ss hh:mm:ss
2 0102 1 4/23/14 17:16:29 4/23/14 17:19:29 00:03:00 3 Light
2 0101 1 4/23/14 17:20:29 4/23/14 17:22:29 00:02:00 2 Light
2 0103 1 4/23/14 17:20:29 4/23/14 17:23:29 00:03:00 3 Light
2 0104 1 4/23/14 17:15:29 4/23/14 17:23:29 00:08:00 8 Light
3 0109 1 4/23/14 17:19:29 4/23/14 17:27:29 00:08:00 8 Light
4 0109 2 4/23/14 17:28:29 4/23/14 17:32:29 00:04:00 4 Light
4 0110 1 4/23/14 17:28:29 4/23/14 17:32:29 00:04:00 4 Light
4 0101 2 4/23/14 17:33:29 4/23/14 17:35:29 00:02:00 2 Light
4 0104 2 4/23/14 17:24:29 4/23/14 17:35:29 00:11:00 11 Light
4 0101 3 4/23/14 17:36:29 4/23/14 17:39:29 00:03:00 3 Light
4 0102 2 4/23/14 17:34:29 4/23/14 17:39:29 00:05:00 5 Light
5 0104 3 4/23/14 17:36:29 4/23/14 17:40:29 00:04:00 4 Light
5 0102 3 4/23/14 17:40:29 4/23/14 17:43:29 00:03:00 3 Light
Detection Parameters
Activity Source
X-Axis
Y-Axis
Z-Axis
Running Wheels
Sleep Threshold
Epochs
2 * 60 sec = 120 sec
Activity Threshold
1 Counts
Analysis Window
Day Start Time Duration
4/23/14 07:00 48:00 hh:mm
Light/Dark Cycle
Light Start Light Duration
07:00 12 Hours
Save configuration
Update
Statistics All
Start Time: 4/23/14 17:13:29
End Time: 4/26/14 08:26:29
Experiment Elapsed Time: 63:13:00
Epochs: 3793
Analysis Window Elapsed Time: 48:00:00
Epochs: 2880
Sleeping Bouts Avg Bout Duration Epochs hh:mm:ss Min Bout Duration Epochs hh:mm:ss Max Bout Duration Epochs hh:mm:ss Total Sleep Time Epochs hh:mm:ss % Sleeping
Light 295 5.003 00:05:00 2 00:02:00 153 02:33:00 1476 24:36:00
Dark 437 4.378 00:04:23 2 00:02:00 26 00:26:00 1913 31:53:00
Total 732 4.630 00:04:38 3389 56:29:00
Help...
Include sleep records in CSV
Select Cages...
Generate CSV File...

Figure 58 - The Sleep Analysis Utility

- **Sleeping Bouts** – Table of calculated sleeping bouts for the currently selected chamber(s) based on the selected settings.
- **Detection Parameters**
  - **Activity Source** – Allows for the selection of specific infrared sensors and running wheels in the calculation of sleeping bouts.
  - **Sleep Threshold** – The conditions for qualifying inactivity as a "sleeping bout".
    - **Epochs** – The number of consecutive samples that have fewer than the specified number of beam breaks to qualify as a bout.
    - **Activity Threshold** – The most beam breaks that can occur in an epoch and still qualify as a sleeping bout.
  - **Analysis Window** – Specifies a period of time within the experiment for analysis.
  - **Light/Dark Cycle** – Specifies the pattern for qualifying bouts as having occurred during the day or at night.
  - **Save Configuration** – Saves the current sleep detection parameters for use with other data.
  - **Update** – Recalculates sleeping bouts based on new settings.
  - **Statistics** – Calculations related to the sleeping patterns of the selected subjects.
  - **Select Cages** – Allows for the selection of specific cages for statistical analysis.
  - **Generate CSV File** – Creates a CSV file contains statistics for each selected subject.
    - **Include sleep records in CSV** – When checked, the sleeping bouts table will be included in the generated CSV.

5.4 Position Analysis

CLAMS systems that are equipped with both X and Y-axis activity sensors have the option of collecting animal position data as well as the standard beam-break data. Columbus Instruments has a range of beam-based activity monitors in different forms but they can essentially be broken down into two product types on the "Oxymax Hardware Status" (**2.2 Hardware Status**)window:

1. **Opto Activity Monitor** – Measures beam-break counts and gives position information about ~0.5-1Hz. The actual sampling rate varies depending on the size and complexity of the system. For stand-alone models, this product is usually branded as the "Opto-M3".
2. **Opto Activity/Position Monitor** – Measures beam-break counts at around the same frequency as the Opto Activity Monitor but can acquire position information as fast as 10Hz. The actual scanning frequency is adjustable within the experiment setup per **3.3.4 Activity Properties**. For stand-alone models, this product is usually branded as the "Opto-M4".

At the conclusion of an experiment, position data can be viewed and exported via the "Position Analysis" utility accessible from the ""File >> Export >> Position Analysis"

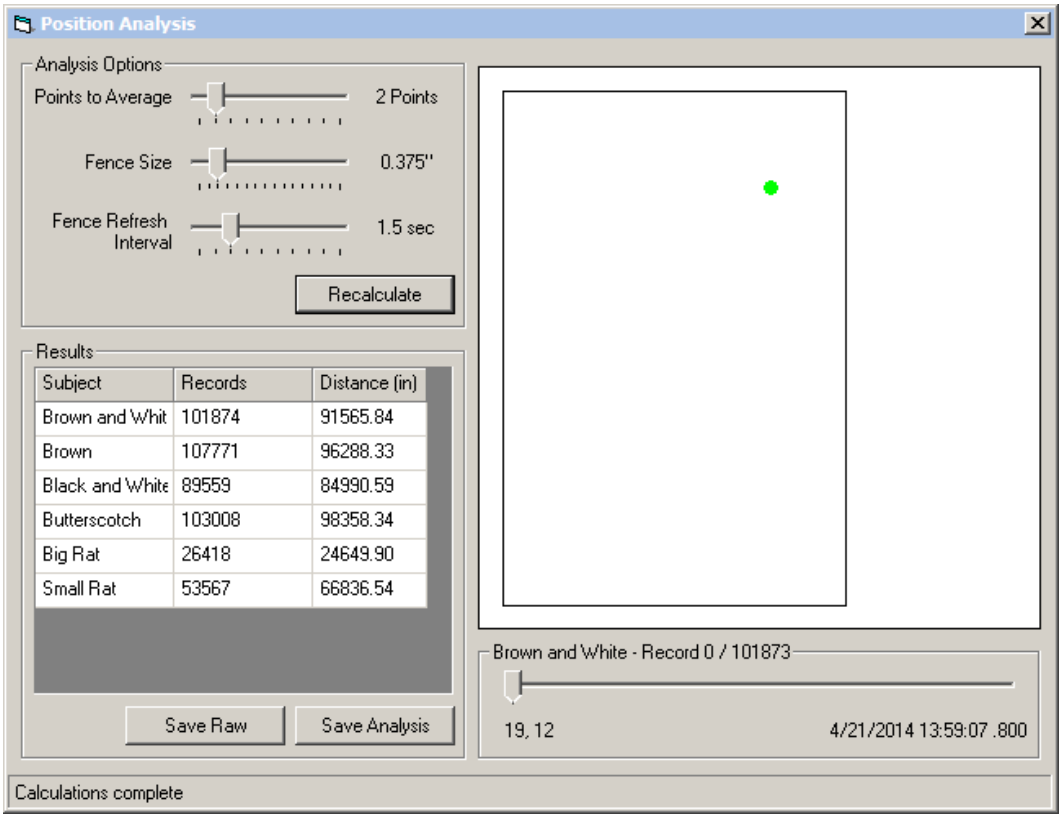


Figure 59 - The Position Export Utility

The position data acquired from the sensors can be exported directly in a "raw" format which consists of every new position record collected during the course of the entire experiment at the selected sampling rate. This raw data includes very small movements that may include rotating and grooming. In general, this raw data is still useful for analyzing time spent in particular zones in the chamber but is less useful for estimating distance travelled. To get a better sense of ambulation the raw data can be filtered to remove some of the stereotypic data.

There are a handful of settings that can be adjusted before exporting the analyzed position data:

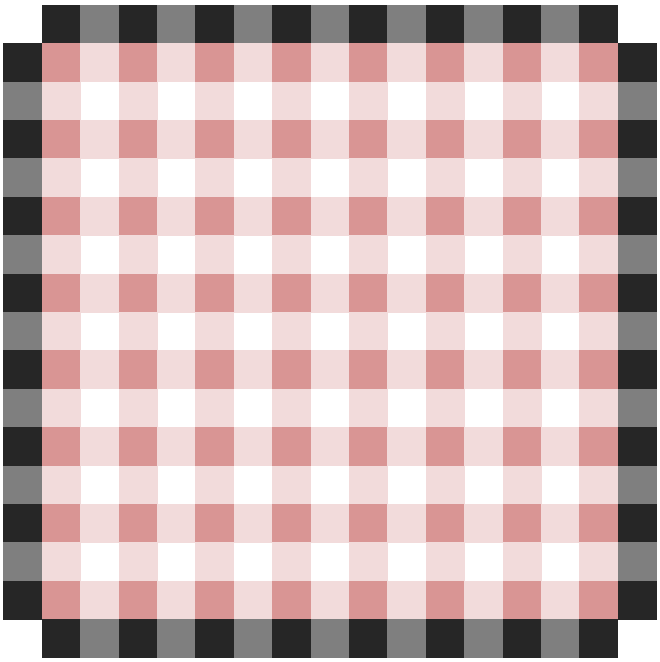
- **Points to Average** – This is the number of successive beam break patterns that are added together to help remove some of the small, rapid movements that don't contribute to distance travelled. Any movements that are repetitive will return as the average position over these points.
- **Fence Size** – Movements centered around a small area usually constitute stereotypic activity. As part of the filtering process, an adjustable "fence" is drawn around the subject's last location. Any movement inside of the fence is not counted against the distance traveled and the animal's location is not updated. Once the subject steps outside of the fence, each new position is considered part of the ambulation until the animal remains still for a particular period of time (the "Fence Refresh Interval").
- **Fence Refresh Interval** – Any time the subject remains still for this period of time the fence is redrawn around the animal.

Once settings have been selected for the export, clicking "Recalculate" will re-analyze the position data for each subject. Chambers with empty data files or chambers without both X and Y-axis sensors will not be analyzed. After analysis, both the raw position data acquired and the analyzed results can be exported as CSV files. The CSV files are generated for all eligible chambers and are automatically saved in the same directory as the rest of the experiment data.

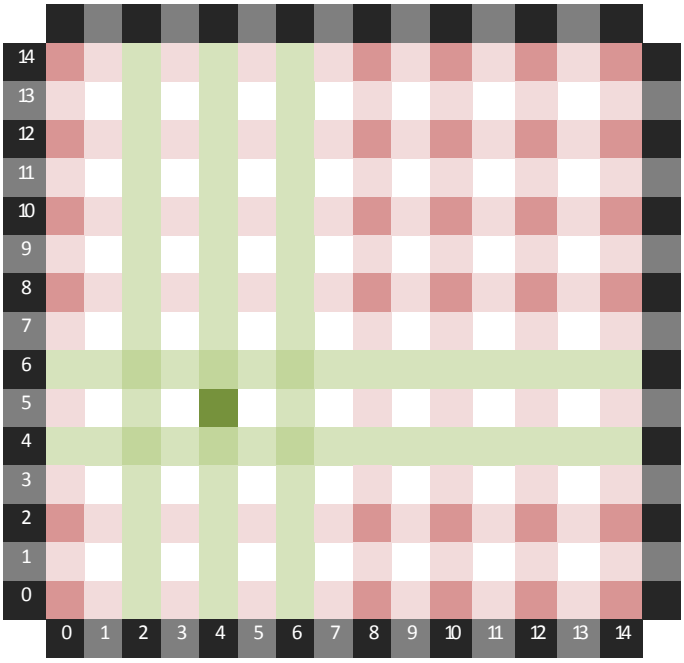
After the data has been analyzed, each position record can be plotted in the space on the right. Select a subject in the table and then use the slider to move through the experiment. The animals location at the printed time appears as a circle. If that record was made as a result of motion, a line indicating the animal's previous position is drawn. The larger circle for the animal's position indicates it was rearing at the time.

5.4.1 – Position Indexes

Position data is presented as a set of coordinates in a unit-less "index" system. The subject's center position on any axis can be calculated to any beam on that axis *or* the space in between beams if an even number of contiguous beams are broken. In this example, there are eight beams – as indicated by **pink lines** – on each axis. The software can calculate the subject's center position to be any of the intersections of the beams – as indicated by the **dark pink lines** – or *any* of the other positions pictured below.



Below is an example of the center position for a subject blocking the **beams marked in green**.



In the above diagram, there are three beams being broken along the Y-axis and two along the X-axis. The **dark green cell** indicates the location of the subject as calculated by the software. The indicated position would be listed as “5, 4”.

Note – Axis notation

Most CLAMS systems mark the long axis on the chamber as the "X-axis". When viewing the chamber from the front, this makes the X-axis run in the direction usually referred to as the Y-axis. For the sake of consistency, this same nomenclature is used in the position data. The position in the lower-left corner of the chamber is referred to as "0, 0". The Y-axis runs left to right; the X-axis runs bottom to top.

There are some situations where the animal is not breaking any beams along one or both of the axes. When this happens the position index is set to "-1". When the animal reappears to break beams a new record will be generated but the distance travelled for that record will always be listed as "zero" since there's no way to discern what the its previous location was.

Appendix A – Oxymax Calculations

A.1 Metabolic Calculations

The metabolic data reported by Oxymax is based on five empirical measurements:

- Gas concentrations measured by sensors:
  - **Reference Oxygen Concentration** ($O2_i$)
  - **Reference Carbon Dioxide Concentration** ($CO2_i$)
  - **Sample Oxygen Concentration** ($O2_o$)
  - **Sample Carbon Dioxide Concentration** ($CO2_o$)
- **Fresh Air Flow**
  - The fresh air flow measured by Oxymax for Windows depends on the ventilation system used. Positive ventilation systems throttle and measure the fresh air before delivering it to the chambers (V_i). Negative ventilation systems draw fresh air into the chamber from the atmosphere around it. The measured flow in this case is not the rate of air entering the cage, but exiting it (V_o).

The calculation of Oxygen consumption (VO_2) and Carbon Dioxide production (VCO_2) values requires the use of both the input (V_i) and output (V_o) flows to the chamber. Despite the fact that the chamber is not pressurized and does not allow gas to build up as an experiment progresses, the chamber cannot be modeled as a perfect steady state system because of the subject's unequal effect on the respiratory gases: the subject does not exhale the same volume of gas as it inhales.

Gases in an Oxymax system are broken into three categories:

- **Consumed** – Oxygen
- **Produced** – Carbon Dioxide, Methane
- **Inert** – All other gases not involved in respiration (Nitrogen, Argon, and other noble gases)

Since inert gases are not used during respiration, their rates of flow at the output (VN_o) and input (VN_i) are the same (Equation 1).

$$VN_i = VN_o$$

Equation 1

The flows of inert gases are products of the flows to and from the chamber and the concentration of inert gases (N_i and N_o) in each flow (Equation 2). The concentrations can be calculated and one of the flows is measured so the other (V_o for positive ventilation, V_i for negative ventilation) can be calculated.

$$V_i N_i = V_o N_o$$

Equation 2

The concentration of inert gases in a sample is assumed to be all gases that are not involved in respiration (Equation 3).

$$N = 100\% - O_2 - CO_2$$

Equation 3

Assuming a positive flow system, combining the previous equations yields the V_o for the system (Equation 4).

$$V_o = V_i \frac{100\% - O_{2i} - CO_{2i}}{100\% - O_{2o} - CO_{2o}}$$

Equation 4

The volumetric flow rates for consumption and production can then both be calculated. The equations are arranged such that both consumption and production are presented as positive values (Equation 5, Equation 6, Equation 7).

$$VO_{2,subject} = V_i O_{2i} - V_o O_{2o}$$

Equation 5

$$VCO_{2,subject} = V_o CO_{2o} - V_i CO_{2i}$$

Equation 6

$$VCH_{4,subject} = V_o CH_{4o} - V_i CH_{4i}$$

Equation 7

Oxymax for Windows always presents consumption and production rates normalized to the subject's body mass in kilograms. To convert the whole-body rates into mass-normalized rates just divide the whole-body rate by the mass (m) of the subject in kilograms (Equation 8).

$$V_{x,norm} = \frac{V_{x,subject}}{m}$$

Equation 8

The accumulated respiration figures (A) are calculated using the whole-body rate and the amount of time that has elapsed since the last measurement (t). The amount of gas consumed or produced in an interval is added to a running total of all sample volumes. Units are adjusted to match the ones specified in the experiment settings (Equation 9).

$$A_x[n] = A_x[n-1] + V_{x,subject} t$$

Equation 9

The respiratory exchange ratio can be calculated from either set of consumption and production rates (Equation 10).

$$RER = \frac{VCO2_{subject}}{VO2_{subject}} = \frac{VCO2_{norm}}{VO2_{norm}}$$

Equation 10

Oxymax for Windows uses one of two possible methods for calculating energy expenditure (Heat). The first uses a "calorific value" (CV) that shows the relationship between heat and the volume of consumed Oxygen. This value is derived from empirical data from Graham Lusk's "The Elements of the Science of Nutrition" (Equation 11).

RER	Kilocalories per liter of O_2
0.707	4.6862
0.75	4.7387
0.80	4.8008
0.85	4.8605
0.90	4.9226
0.95	4.9847
1.00	5.0468

$$CV = 3.815 + 1.232 * RER$$

Equation 11

The rate of energy expenditure can then be calculated using the subject's rate of Oxygen consumption (Equation 12). This equation is standard for small-to-medium, non-ruminant mammals.

$$Heat = CV * VO_{2,subject}$$

Equation 12

Oxymax for Windows also allows for the definition of a custom heat calculation equation. Coefficients for Oxygen, Carbon Dioxide and Methane rates can be specified and used to calculate heat (Equation 13).

$$Heat = (K_1 * VO_{2,subject}) + (K_2 * VCO_{2,subject}) + (K_3 * VCH_{4,subject})$$

Equation 13

A.2 "Analysis of the Oxidation of Mixtures of Carbohydrate and Fat"

The following is an excerpt from a correction issued by Graham Lusk in 1923 that was published in the journal, "Animal Calorimetry". It contains the table of oxidation figures for carbohydrates and fats and the equation used by Oxymax for calculation of Heat.

Equation 14 shows the original form of the equation shown as "(5)" in Figure 60 which simplifies to the previously mentioned Equation 11.

In 1901 Zuntz and Schumburgl (Zuntz, N., and Schumburg, Studien zu einer Physiologie des Marsches, Berlin, 1901, 361.) published a standard table showing the caloric value of a liter of oxygen when used to oxidize mixtures of carbohydrate and fat. Figures were given for respiratory quotients varying from 1.00, for pure carbohydrate, to 0.707, for pure fat. The writer elaborated this table (Williams, H. B., Riche, J. A., and Lusk, G., J. Biol. Chem., 1912, xii, 357. Lusk, G., The elements of the science of nutrition, Philadelphia, 3rd edition, 1917, 61.) and introduced columns showing the relative quantity in calories of carbohydrate and fat consumed. That these figures were in error was pointed out to me by Dr. H. H. Mitchell in a letter dated June 26, 1917, and the error has since been called to my attention by my students. To one of them, Mr. A. M. Michaelis, I am indebted for the following corrected table. Although the error is not great, yet it is worthy of note and of record. The table serves to illuminate the charts recently prepared by Dr. E. F. Du Bois, which he presents in the article immediately following this.

$$CV = 4.686 + \frac{RER - 0.707}{0.293} * 0.361$$

Equation 14

$$CV = 3.815 + 1.232 * RER$$

TABLE I.

Analysis of the Oxidation of Mixtures of Carbohydrate and Fat.

R. Q.	Percentage of total oxygen consumed by:		Percentage of total heat produced by:		Calories per liter O_2.	
	Carbohydrate. (1)	Fat. (2)	Carbohydrate. (3)	Fat. (4)	Number. (5)	Logarithm. (6)
0.707	0	100.0	0	100.0	4.686	0.67080
0.71	1.02	99.0	1.10	98.9	4.690	0.67114
0.72	4.44	95.6	4.76	95.2	4.702	0.67228
0.73	7.85	92.2	8.40	91.6	4.714	0.67342
0.74	11.3	88.7	12.0	88.0	4.727	0.67456
0.75	14.7	85.3	15.6	84.4	4.739	0.67569
0.76	18.1	81.9	19.2	80.8	4.751	0.67682
0.77	21.5	78.5	22.8	77.2	4.764	0.67794
0.78	24.9	75.1	26.3	73.7	4.776	0.67906
0.79	28.3	71.7	29.9	70.1	4.788	0.68018
0.80	31.7	68.3	33.4	66.6	4.801	0.68129
0.81	35.2	64.8	36.9	63.1	4.813	0.68241
0.82	38.6	61.4	40.3	59.7	4.825	0.68352
0.83	42.0	58.0	43.8	56.2	4.838	0.68463
0.84	45.4	54.6	47.2	52.8	4.850	0.68573
0.85	48.8	51.2	50.7	49.3	4.862	0.68683
0.86	52.2	47.8	54.1	45.9	4.875	0.68793
0.87	55.6	44.4	57.5	42.5	4.887	0.68903
0.88	59.0	41.0	60.8	39.2	4.899	0.69012
0.89	62.5	37.5	64.2	35.8	4.911	0.69121
0.90	65.9	34.1	67.5	32.5	4.924	0.69230
0.91	69.3	30.7	70.8	29.2	4.936	0.69339
0.92	72.7	27.3	74.1	25.9	4.948	0.69447
0.93	76.1	23.9	77.4	22.6	4.961	0.69555
0.94	79.5	20.5	80.7	19.3	4.973	0.69663
0.95	82.9	17.1	84.0	16.0	4.985	0.69770
0.96	86.3	13.7	87.2	12.8	4.998	0.69877
0.97	89.8	10.2	90.4	9.58	5.010	0.69984
0.98	93.2	6.83	93.6	6.37	5.022	0.70091
0.99	96.6	3.41	96.8	3.18	5.035	0.70197
1.00	100.0	0	100.0	0	5.047	0.70303

Formula for Column (R. Q. = R)

(1) $\% = 100\,\frac{R - 0.707}{0.293}$

(2) $\% = 100\,\frac{1.00 - R}{0.293}$

(3) $\% = \frac{504.7\ (R - 0.707)}{5.047\ (R - 0.707) + 4.686\ (1.00 - R)}$

(4) $\% = \frac{468.6\ (1.00 - R)}{5.047\ (R - 0.707) + 4.686\ (1.00 - R)}$

(5) $\text{Calories} = 4.686 + \frac{R - 0.707}{0.293} \times 0.361$

(6) Logarithm = log of Column 5

Figure 60 - Oxidation Table

Appendix B – Warning Log Messages

During an experiment, non-critical message may be logged to the "Warning Log" tab which should be checked occasionally. The following is a list of messages and descriptions for messages current as of Oxymax for Windows v5.10+.

- **Lost serial communication on COM**
  - The serial port Oxymax was using for communication was disconnected from Windows. This is usually the result of the USB cable or adapter being removed from the computer.
- **Serial communication restored on COM**
  - The serial port Oxymax was using before it was disconnected has been reconnected.
- **O2 Out outside of normal bounds**
  - The value read for O2 Out exceeds the normal bounds for an animal based on the O2 In value. This can happen because the concentration from the chamber is lower than the sensor can read or because the read differential is in excess of what is healthy for the animal.
- **CO2 Out outside of normal bounds**
  - The value read for CO2 Out exceeds the normal bounds for an animal based on the CO2 In value. This can happen because the concentration from the chamber is lower than the sensor can read or because the read differential is in excess of what is healthy for the animal.
- **CO2 sensor reached upper limit**
  - Detected CO2 values are at the maximum detectable level for the sensor. DO2/VCO2 readings will not be correct. This usually happens when either the sensor has malfunctioned or fresh air has been set far lower than is necessary.
- **High Sample Humidity**
  - When a system has an Environmental Monitor, this indicates that the read Sample humidity is too high. This usually is the result of the Drier running out of desiccant or the drier tube failing.
- **High Reference Humidity**
  - When a system has an Environmental Monitor, this indicates that the read Reference humidity is too high. This usually is the result of the Drier running out of desiccant or the drier tube failing. This channel is usually only enabled for systems with Zirconia Oxygen sensors.
- **High Purge Humidity**
  - This indicates that the desiccant used in the tube-drier has been depleted.
- **Fresh Air Flow out of tolerance**
  - The measured fresh air for this chamber – or group of chambers – is more than 10% away from the target fresh air value. Depending on the type of flow controller, the issue could be the result of a clog, a leak, or having selected a flow higher than is capable given the pump and restrictors in use.

- **VDM Water Reservoir Empty**
  - The VDM unit has detected that the water reservoir is empty and needs to be refilled otherwise no subjects will be able to drink.
- **VDM Overpump Warning; pumping too much water**
  - The indicated channel has pumped more than the set limit within the given interval. This usually indicates a fault of the sipper or the wiring where water is being detected as consumed and replaced at a rate too fast for a subject to actually consume.
- **VDM Failure to Drink**
  - The rate of water consumption for a particular subject was lower than the set limits. Occasionally, this can be because the subject really isn't thirsty but it can also indicate an issue with the sipper where the tip becomes occluded and the subject can no longer drink.
- **Zirconia Reference Voltage differential exceeds limits**
  - During reference readings, the Zirconia Oxygen sensor checks the voltage indicated while the same gas – room air – is being measured on both sides of the sensor. Under stable conditions, this voltage remains largely unchanged. If the new value is noticeably different than the last value this message will appear. It usually just indicates a noticeable change in room concentration as a result of people entering or leaving the room. If this item is logged a couple of times a day, it does not indicate an issue. If it occurs after every reference – 30+ times a day – then there may be an issue with sample flow or the sensor itself.
- **Zirconia Reference Voltage out of bounds**
  - The reference voltage has risen or fallen to the limits of the sensors ability. This would usually occur if the actual calibrated position for the sensor was set incorrectly and the reference voltage moved just a little more out of bounds.
- **O2 In value changed significantly between references**
  - The O2 In value changed more than 0.02% between readings. If this happens frequently, the room could be too unstable or poorly ventilated. Increase referencing frequency to make sure the changes are smaller.
- **CO2 In value changed significantly between references**
  - The CO2 In value changed more than 0.005% between readings. If this happens frequently, the room could be too unstable or poorly ventilated. Increase referencing frequency to make sure the changes are smaller.

Appendix C – Diagram for Oxygen Fuel Cell Replacement

Figure 61 illustrates the proper assembly of an Oxygen Fuel Cell in a standard cell housing. It is essential that the provided O-ring is secured to the replacement Fuel Cell before inserting it into the housing.

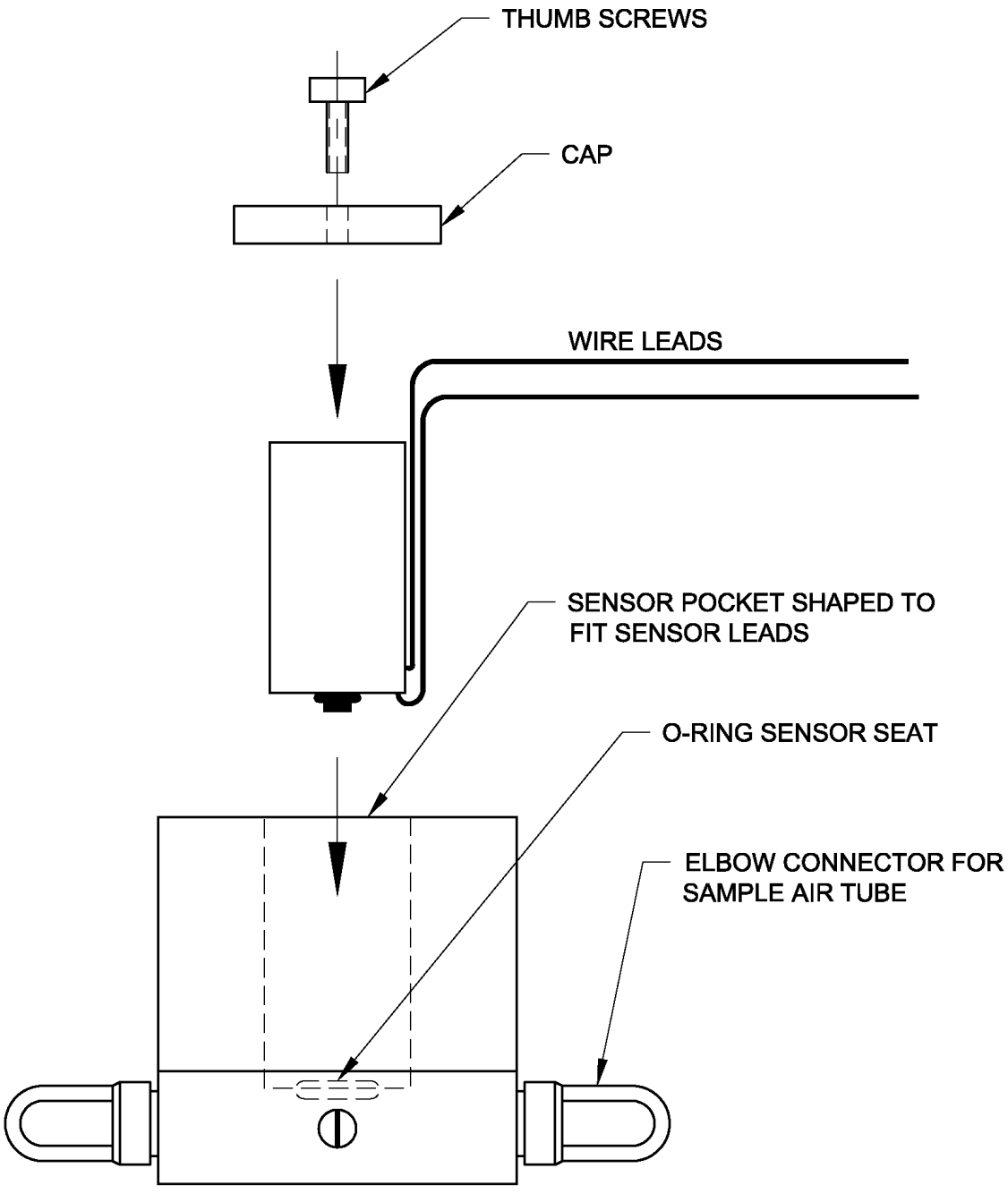


Figure 61 - Fuel Cell Assembly

Appendix D – Procedure for Changing Ammonia Traps

Components inside the Gas Sample Drier and Dual-Tube Drier products are very sensitive to the ammonia in urine. Ammonia needs properly scrubbed from any sample passed through the driers to prevent damage. The ammonia is removed from incoming samples via traps packed with copper sulfate like those pictured below.

The copper sulfate will turn dark blue and/or aqua-green depending on the concentration of ammonia passing through it. The best practice for attaching and maintaining ammonia filters is as follows:

- Ammonia traps should be connected two-at-a-time in series right before a particulate filter as pictured above.
- The location of this filter assembly will vary by system configuration but is usually the last component connected to the vacuum side of the sample pump. The drier is usually the first component on the sample pump's pressure side.
- When the system is in use, the first ammonia trap will change color depending on the ammonia saturation. The exact color of the expended portion of the trap is not important. A completely expended ammonia trap is pictured below.

- When the second ammonia trap begins to exhibit *any* color change, it means that the first trap is expended and needs to be replaced.
- It is best to replace the ammonia trap in between experiments but it can be exchanged during an experiment in an emergency. If the trap is being changed while an experiment is running, try to perform the exchange during a reference reading and be sure to mark an Event.
- To replace the expended trap, remove it from the assembly and shift the old second trap into the first trap position.
- Put the brand new ammonia trap into the second trap position.

Appendix E – Replacing Hydroscopic Tubing in Sample Drier

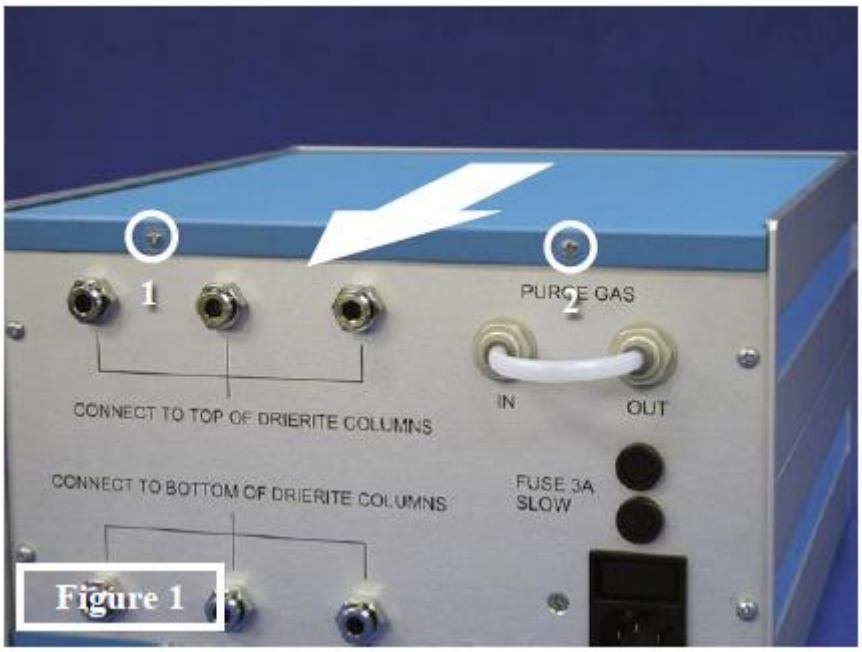


Figure 1

Remove the two screws from the lid as in Figure 1 and slide the lid off to reveal the inside of the cabinet as shown in Figure 2. The Tube Drier is held in place by three white clamps, indicated in Figure 3. Two are located on the side in plain view, and the third is mounted vertically on the rear panel beneath the stainless steel fittings for the desiccant columns.

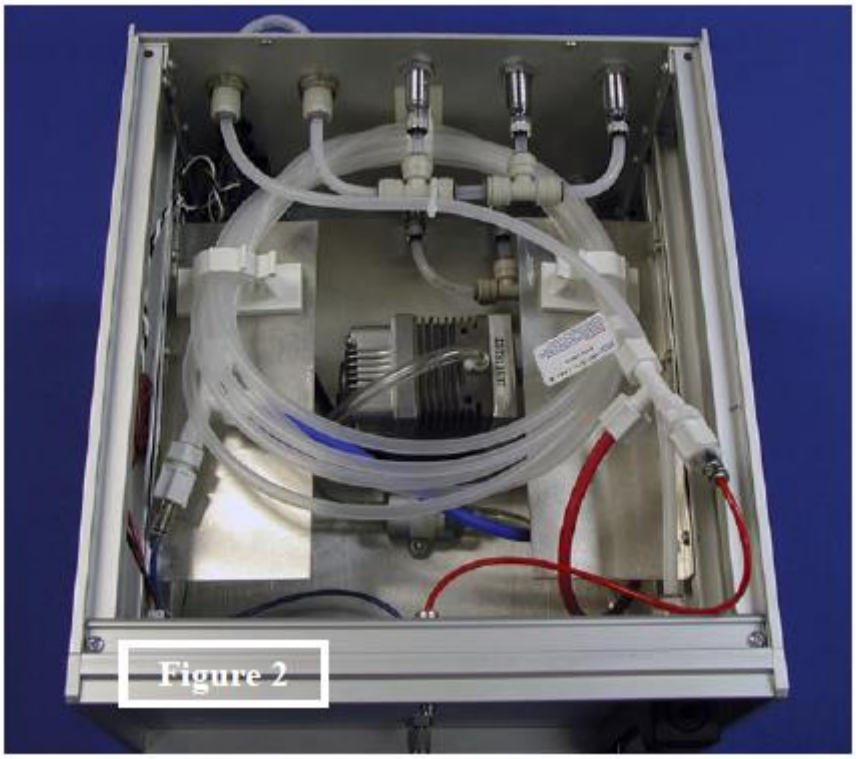
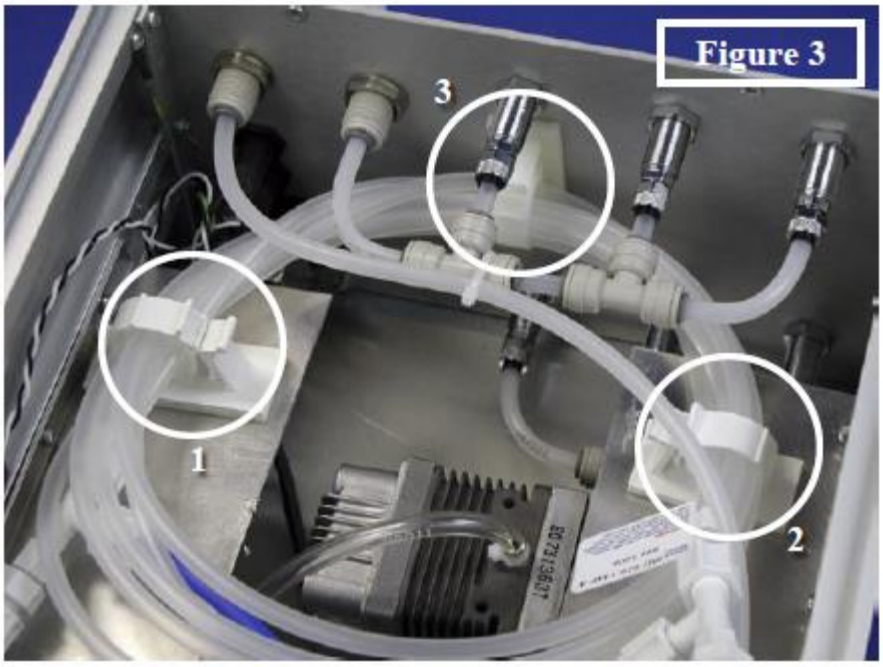
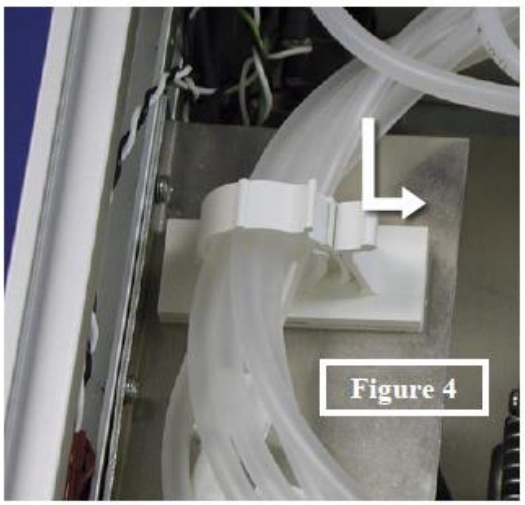
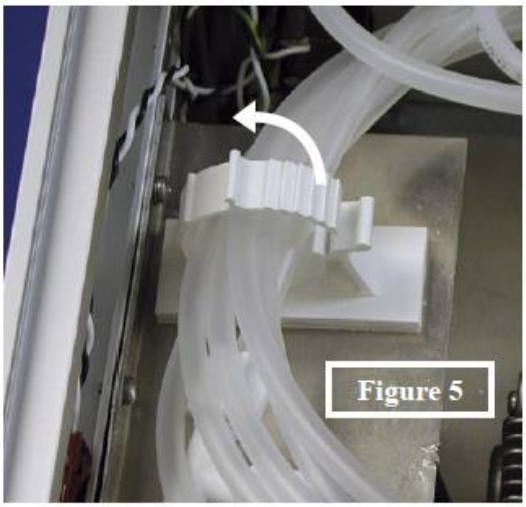


Figure 2

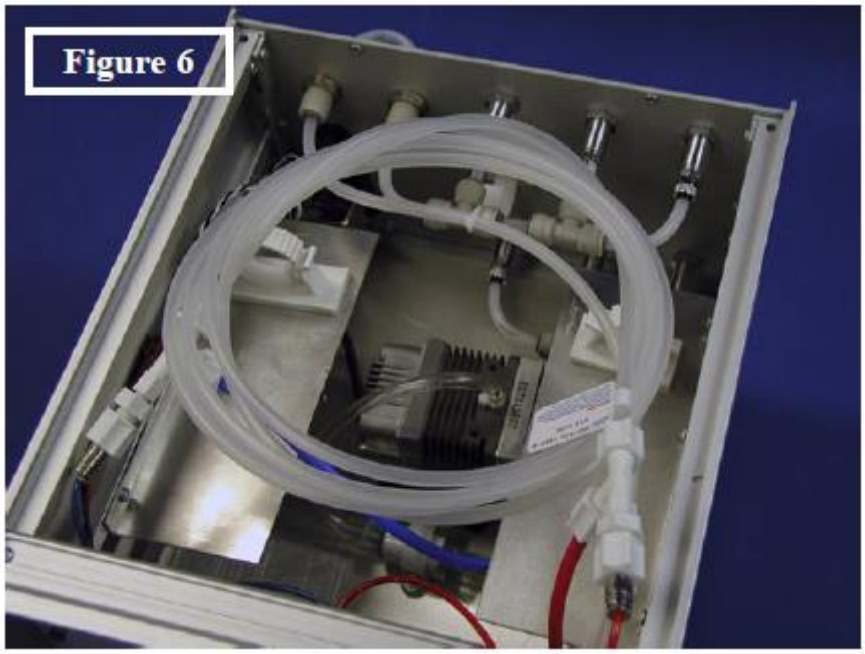


To unlock the clamps, push down on the tab then pull it away slightly from the round clamp. The round clamp will naturally loosen itself partially; you may need to pull it free of the tab in order to completely open the clamp. Figures 4 and 5 show the directions in which to manipulate the clamp in order to open it completely.



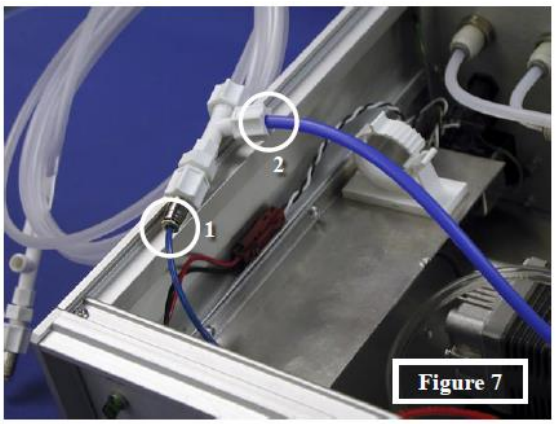


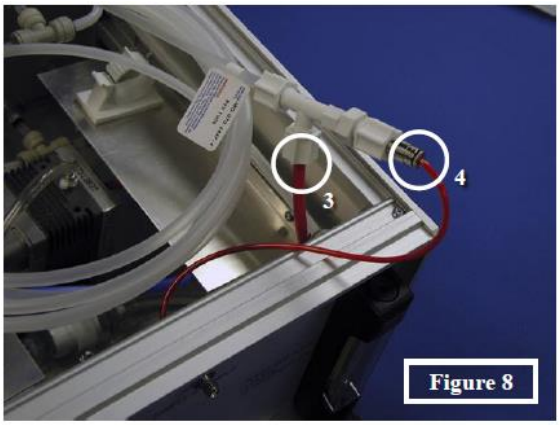
Carefully remove the tube drier from the clamps so that it lays loosely inside the cabinet as pictured in Figure 6.



Disconnect the tube drier at the four fittings shown below. There are two types of fittings on each side; a red 1/8 inch quick-connect fitting, a red 1/4 inch screw fitting, and a duplicate set on the blue side. The screw fittings simply unscrew to disconnect. To disconnect from the quick-connect fitting, compress the ringed collar against the fitting itself, holding it in while pulling the tube out.

This procedure is easiest performed by doing one side at a time. Typically the red side first, then rotating the tube assembly (as pictured in Figure 7) outside the cabinet to more easily access the blue tube fittings.





Quick Connect Fittings: To release the colored nylon tube, push in on the fitting's collar indicated by the black arrows below. While holding the collar 'in" you can pull the tube out of the fitting. The tube will not release unless the collar is completely pushed in.

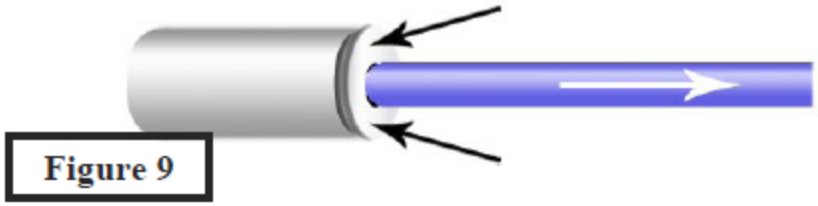
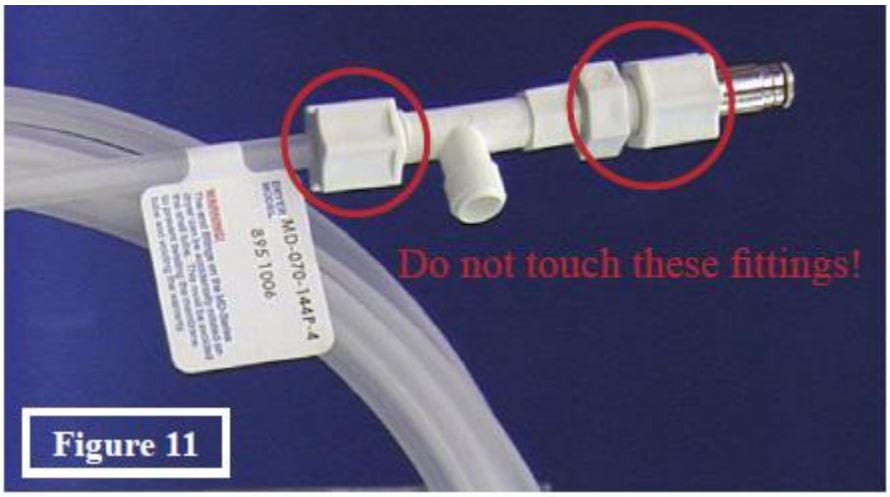


Figure 9

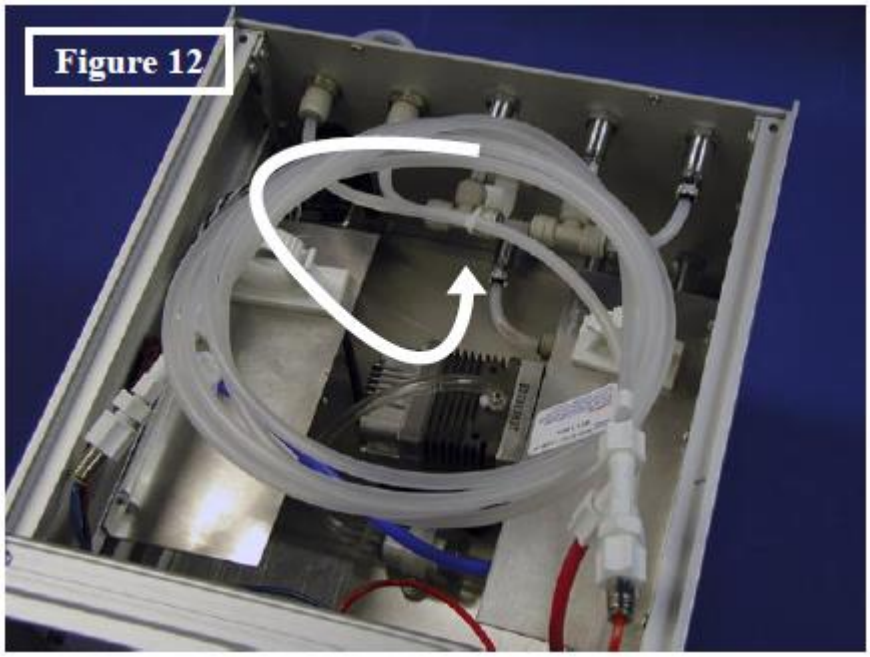
Figure 10

Remove the new replacement tube drier from the packaging. Reverse the preceding steps to install the new tube. First attach the colored tubes to the fittings, then secure the tube drier in place with the white clamps. The screw-fittings need only be "finger tight," no tools should be used. To insert the tube into the quick connect fitting, simply push the tube into the hole. The collar does not need to be pushed in.

Please note that the white fittings circled below should neither be tightened nor loosened. Loosening will cause a leak, tightening it will crack the Nafion material, thus causing a leak.



When securing the tube drier and placing it into the white clamps, it is critical to not twist or kink the tube. This will damage the Nafion tubing and possibly create a leak. It's easiest to first secure the tube in the vertically mounted white clamp in the back. The tube assembly must be lifted slightly and moved toward the front of the cabinet in order to slide the tube drier under the stainless steel fittings. Once the rear clamp is secured, the two sides will lay almost naturally into the remaining clamps. Secure these and place the lid back onto the cabinet, securing it with the two screws removed at the beginning of these instructions.



Appendix F – Environmental Enclosure Light Controller Setup

The following are the instructions for the first time setup of the Intermatic Incubator Light Controller.

1. Press the “Reset” button.
2. Set the time format to 24-Hour or AM/PM by using the +/- buttons and press OK to select.
3. Set the current hour using the +/- buttons and press OK to select.
4. Set the current minute using the +/- buttons and press OK to select.
5. Select current day of the week using the +/- buttons and press OK to select.
6. Check the failsafe dials in the refrigeration cabinet.
    a. The Low Failsafe should be at 0.
    b. The High Failsafe should be at 50.
7. Set P01 (referenced on Page 10 of the Intermatic Instruction Manual).
    a. Depress the reset button.
    b. P01 should blink.
    c. Press OK.
    d. Select P01 and press OK.
    e. Use +/- to select the hour to turn on and press OK to select.
    f. Use +/- to select the minute to turn on and press OK to select.
    g. Use +/- to select the hour to turn off and press OK to select.
    h. Use +/- to select the minute to turn off and press OK to select.
    i. Use +/- to select what days to use P01 and press OK to select.
8. Select “Auto”.
9. The light cycle should begin at this point.

Appendix G – Calibrating Modular Treadmills

Current models of the Columbus Instruments Metabolic Treadmill can be recalibrated in the field by use of the included speed sensor. Calibration of the treadmill belt speed should be performed every three months. It is also helpful to calibrate the unit whenever switching between species.

1. Ensure the Treadmill Controller front panel toggle is set to "STOP".
2. Place speed sensor on treadmill belt and fasten it with the screw nut as shown.

3. Using the dial on the Treadmill Controller, adjust the speed to 88.7 m/min.
4. Set the electric stimulus frequency to 2 Hz on Treadmill Controller front panel.
5. Push the "ODOMETER RESET" button to initialize calibration procedure. The LCD on the front panel will read "AutoCal Speed" as shown.

6. Push the "ODOMETER RESET" button a second time to advance to belt calibration start. The LCD on the front panel will read "Run to Start…" as shown.

7. Set toggle to "RUN" to begin the series of belt speed and acceleration tests.
8. Upon belt calibration completion, the most recent calibration is automatically saved, and speed sensor counts (OD counts) are displayed. Obtaining count values between 4000-5000 indicates calibration was successful.

9. To exit the calibration, press the "ODOMETER RESET "button. A "Calibration Saved" message will be displayed as a further reassurance that the most recent calibration was saved automatically. To complete the process and easily return to the main menu, simply power the unit off and then back on using the switch located on the rear of the controller.

Appendix H – Oxymax Hydrogen Sensor

H.1 Overview

The Columbus Instruments Hydrogen Sensor for metabolic applications is a fuel cell type measurement device operating under many of the same principles as the Fuel Cell Oxygen Sensor. A chemical reaction in the cell produces electricity that varies with the amount of Hydrogen present in the cell.

- Operating Range – 0 to 2000ppm
- Expected Operating Life – 2 Years
- Temperature Range – -20^{0}C to +50^{0}C
- Relative Humidity Range – 15 to 90%, non-condensing

Additionally, it should be noted that the chemical fuel cell will dry out and expire if kept in exceptionally dry environments for extended periods of time. This is counter to the best-practices regarding other gas sensors which function best in perfectly dry environments. It is for this reason that the Hydrogen sensor is plumbed in after the sample pump and before the drier unit.

H.2 Components and Installation

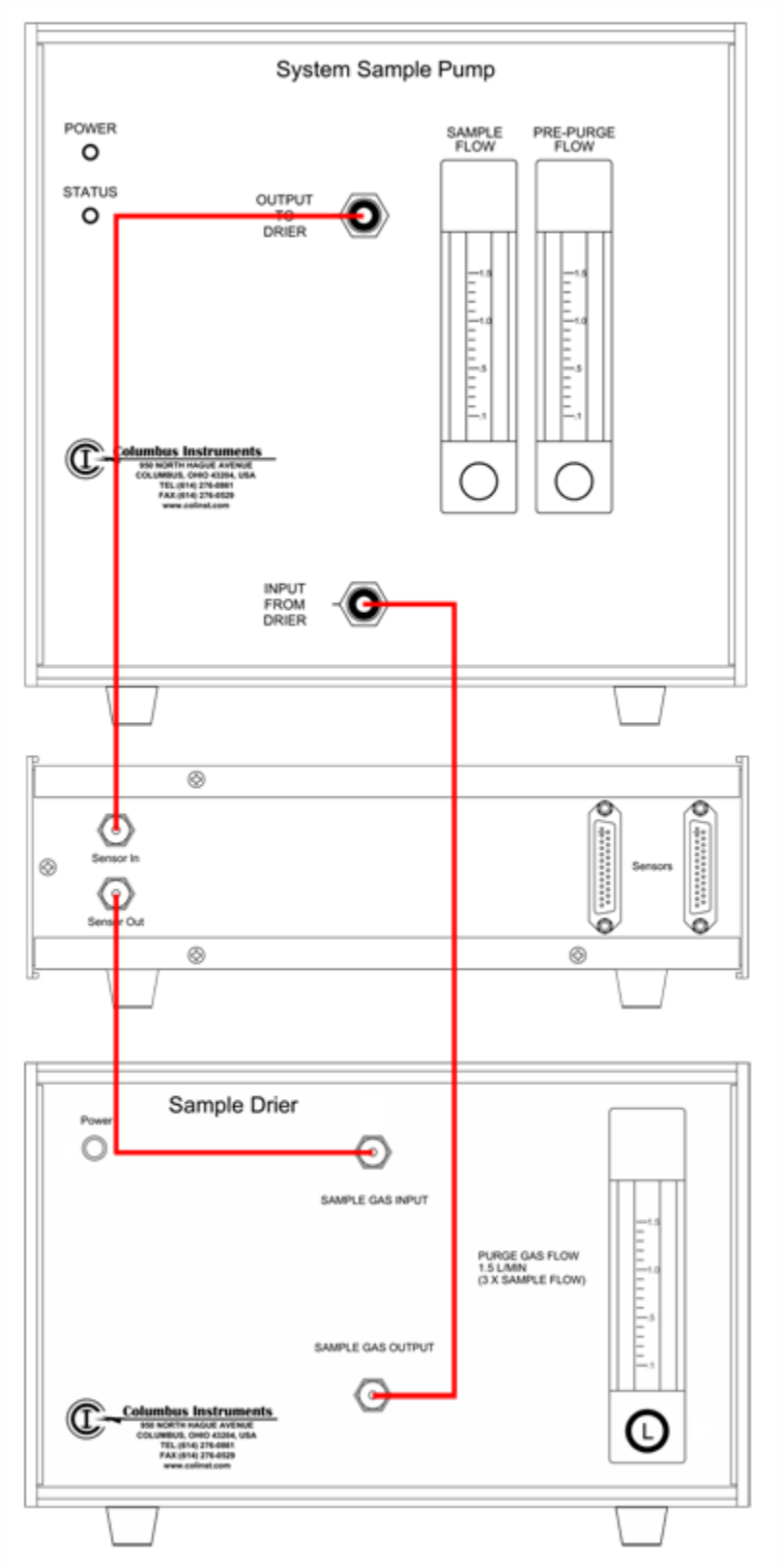
The only components required for installing a Hydrogen Sensor into a functioning Oxymax system are:

- Hydrogen Sensor
- A few feet of 1/8" tubing
- Extra nuts and ferrules
- 25-pin serial cable

In fact, it's likely that the Hydrogen Sensor's fittings will already include the requisite nuts and plastic ferrules.

There are two connections that need to be made for the Hydrogen Sensor to function: electrical and gas.

With regard to the tubing connection required for the sample gas, the Hydrogen sensor will receive the sample immediately after the sample pump and before it is dried by the Sample Drier as shown below.


System Sample Pump
POWER
STATUS
OUTPUT TO DRIER
SAMPLE FLOW
PRE-PURGE FLOW
Columbus Instruments
INPUT FROM DRIER
Sensor In
Sensor Out
Sensors
Sample Drier
Power
SAMPLE GAS INPUT
PURGE GAS FLOW
1.5 L/MIN
(3 X SAMPLE FLOW)
SAMPLE GAS OUTPUT
Columbus Instruments

Before installing the Hydrogen Sensor, the top-most fitting on the front of the System Sample Pump ("Output to Drier") connects directly to the top-most fitting on the front of the Sample Drier ("Sample Gas Input") and the bottom fitting on the Sample Drier ("Sample Gas Output") connects back to the System Sample Pump ("Input from Drier"). This second connection will remain untouched as long as tubing lengths and device placement allows for it.

To add the Hydrogen Sensor to the sample line:

1. Disconnect the tube from the front of the Sample Drier's "Sample Gas Input" port.
2. Connect this now-loose tubing to the back of the Hydrogen Sensor at the port labeled "Sensor In". If this piece of tubing is not long enough, cut a new piece of tubing from the extra provided to the appropriate length and replace the existing tube.
3. Cut a piece of 1/8" tubing long enough to connect the Hydrogen Sensor to the Sample Drier.
4. Connect this new piece of tubing from the "Sensor Out" port on the back of the Hydrogen Sensor" to the "Sample Gas Input" on the front of the Sample Drier.

To connect the 25-pin serial cable that functions both a power cable and communication line:

1. Power off the entire system.
2. On the back of the Carbon Dioxide Sensor, there are two 25-pin serial ports with cables attached. One of these cables leads to the Oxygen Sensor: disconnect that cable from the back of the Carbon Dioxide Sensor.
3. Connect one end of the provided 25-pin serial cable to the now-vacant port on the back of the Carbon Dioxide Sensor and connect the other end to either of the free ports on the back of the Hydrogen Sensor.
4. Connect the loose-end of the Oxygen Sensor's serial cable to the remaining open port on the back of the Hydrogen Sensor.
5. Tighten the thumb-screws on the ends of each cable to ensure solid connections.
6. Turn the power to the system back on.

H.3 Experiment Setup

H.3.1 Overview

It's worth noting at this time that the Oxymax calorimeter for animals was designed to only natively support *three* gases and it is only through its scalability and a few tweaks that additional gases are supported. It is because of this that Hydrogen Sensor calibration and data collection require a few additional steps as the software does not support it natively. Understanding the data collected from a four-gas system is covered in **H.4 Reading Data**.

It must also be noted that the Hydrogen Sensor is only supported by Oxymax for Windows version 4.51 or later. If you have received an updated installation disc or configuration files with the Hydrogen Sensor, be sure to install the latest version of each before proceeding.

H.3.2 Pre-Experiment

Unless otherwise instructed, typical warm-up times for an Oxymax system from power-on to calibration range from about one to three hours. However, the Hydrogen Sensor requires *eight hours* of power before it can be calibrated. It is therefore advises that the system be powered on and the software initialized the evening before the start of a scheduled experiment.

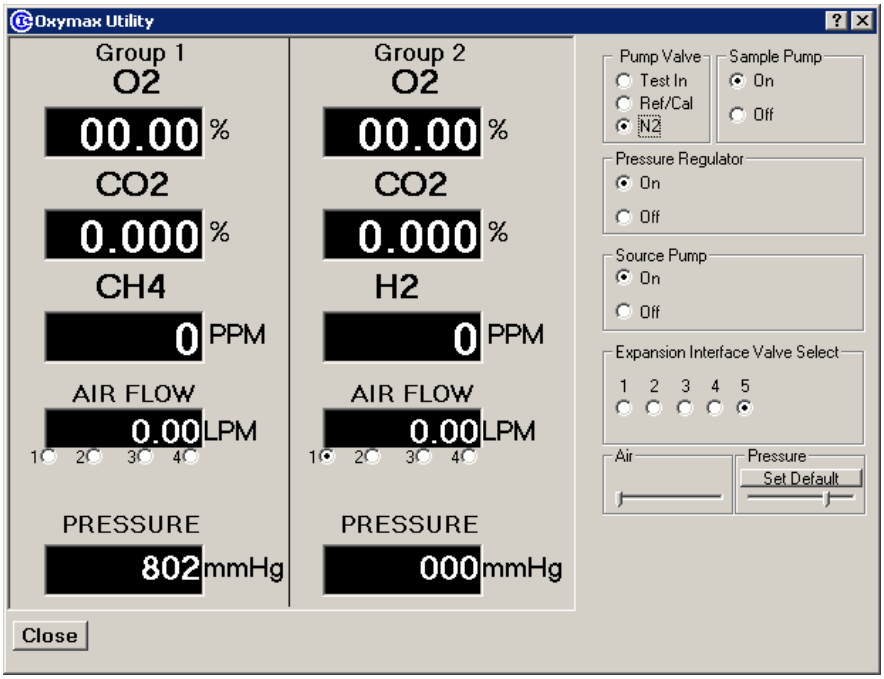
After the software has been initialized and the sample pump is turned on, it's important to check the sample flow on the front of the System Sample Pump. The red ball in the flow meter that marks the sample flow should hover directly between the marks indicating 0.4 and 0.5 LPM. This indicates a sample flow of about 0.45 LPM.

H.3.3 Calibration

After the system has been turned on and the software has been initialized for at least eight hours, the Hydrogen Sensor is ready to be calibrated. Since there is no built-in calibration routine for the Hydrogen Sensor, it must be done manually using the Oxymax Utility. The Oxymax Utility can only be accessed before opening an experiment configuration so it must be done first.

To calibrate the Hydrogen Sensor:

1. Select "Oxymax Utility" from the "Tools >> Utilities" to open the window shown.



2. Notice that there are now *two* columns of gas sensor data in the Oxymax Utility where there was only one before. The first column contains current readings from

the Oxygen, Carbon Dioxide and Methane sensors. The second contains identical copies of the Oxygen and Carbon Dioxide data but shows the Hydrogen Sensor in place of the Methane Sensor in this group.

3. At this point the Oxygen, Carbon Dioxide and Methane sensors have not been calibrated so their readings may be considerably off from room air.
4. The first step to calibrating the Hydrogen Sensor is establishing the proper offset point. For this, we require a gas without Hydrogen in it. Systems that employ Paramagnetic Oxygen Sensors are already equipped with a tank of pure Nitrogen which will provide a Hydrogen offset point. Systems without Nitrogen tanks can simply use regular room air as Earth's atmosphere around sea level is only 1 ppm (parts per million) of Hydrogen or less.
5. In either case, start flushing the Hydrogen Sensor by clicking the "N2" option under the "Pump Valve" heading in the upper-right corner of the Oxymax Utility. This will either flush the sensor with Nitrogen or with CO2 free air from the Soda Lime column.
6. All the system to move air through the sensor for at least three minutes; it may be helpful to have a timer present.
7. At the end of the three minute period, observe the Hydrogen reading. Slowly adjust the "Offset" knob on the front of the sensor itself to move the H2 reading to 0 ppm. If the display read 0 ppm to begin with, it's advisable to slowly turn the knob clockwise until the value reads above 0 ppm. Then, slowly turn the knob counter-clockwise until the value just barely switches back to 0 ppm.
8. Use the black switch on the perimeter of the Offset knob to lock the current value into place.
9. Under the "Pump Valve" heading in the Oxymax Utility, now select the "Ref/Cal" option.
10. If your system has multiple tanks of Calibration Gas, be sure that the Hydrogen Calibration Gas is connected to the calibration line.
11. Turn the Calibration Gas tank on and check the overflow line at the base of the calibration line for overflow. A small, but perceivable excess of gas should be noticeable from this tube.
12. Once the gas is on, wait at least three minutes for the system to flush the old gas out of the Hydrogen Sensor and replace it with Calibration Gas.
13. At the end of the three minute period, slowly adjust the "Gain" knob on the front of the unit until the value for Hydrogen on the screen matches the concentration of Hydrogen in the Calibration Gas. This value can either be found on a label on the tank or with a separate certificate.
14. Use the black switch on the perimeter of the Gain knob to lock the current value into place.
15. Turn off the calibration gas.
16. Close the Oxymax Utility.

Now continue setting up and preparing for an experiment as you normally would. The calibration procedures for the Oxygen and Carbon Dioxide Sensors will prompt you to

calibrate two sets of sensors, just remember that both displays are actually tied to the same set of sensors. When calibrating the Methane sensor, the Methane calibration procedure should work properly for the "Group 1" sensor but will list calibration errors for "Group 2". Ignore the errors regarding Group 2. The knobs on the Hydrogen Sensor should not be adjusted at this time regardless of the software's prompts.

When setting up Subject properties on the "Experiment >> Setup" screen, notice that twice as many subjects appear as there are channels in the system. For every subject (example "Subject 1") it appears in both channel 0101 and 0201. Both channels must be enabled in order to collect samples from all sensors properly. Additionally, the calculation of production and consumption rates to work out properly, any changes in flow must also be made to *both* entries for a subject as well. For example, when adjusting the flow in Chamber 2 to something like 5 LPM, both 0102 and 0202 must read "5".

Due to the comparatively long response time of the Hydrogen Fuel Cell the settle times for both reference and cage measurements needs to be set to at least **three minutes** instead of the usual one to two minutes.

H.4 Reading Data

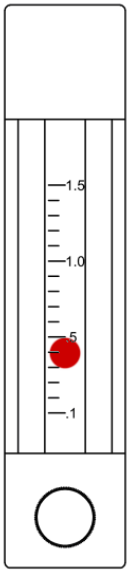
In order to add support for a fourth gas sensor to Oxymax, the hardware configuration files have been modified to show *two* chambers for every one chamber that actually exists in the system. Each actual chamber then has two entries for every measurement. For each chamber "XX", there will be two sets of measurements listed for channels 01XX and 02XX. Many of the listed measurements will be duplicated with very small variances; the following table indicates the locations in the dataset where each gas is recorded.

Gas	Channel
Oxygen	01XX
Carbon Dioxide	01XX
Methane	01XX
Hydrogen	02XX

The other base-metabolic measurements should all be taken from the "Group 1" (01XX) channel for each chamber.

Appendix I - Quickstart Experiment Checklist

- Power on the CLAMS system
  - Check "Power" LED's for all boxes
  - **Feeding, Urine** – Turn on all balances
    - **Feeding** – Put feeders on balances before powering them on
- Run Oxymax for Windows to start warm up
  - Select hardware configuration
  - Allow software to initialize hardware
- Allow system to warm-up
  - About one to two hours
  - **Zirconia O_2 Sensor** – Wait for temperature to reach ~725 degrees C
  - **Automatic CO_2 Sensor** – Wait for blue or green "Ready" light (should take ~20-30 min)
  - **Feeding** – Fill feeders
  - Fill water bottles
    - **Volumetric Drinking Monitor** – Check liquid reservoir and make sure detection clips are secure to the sippers
  - Place subjects in applicable chambers
    - Lids should clasp firmly and evenly
  - Check nut-and-ferrule fittings on lids to ensure a tight fit
  - If the system adapts to both mice and rats, check plastic flow restrictors in fresh air lines
    - All restrictors should be present, regardless if the chamber will be used in the current experiment
    - All restrictors should be the same color
  - If the system's sample flow is controlled manually, set to ~0.40 LPM as shown



- After warm-up period, calibrate gas sensors
  - "Tools >> Calibrate"
  - Set CalGas span values to Oxygen% and Carbon Dioxide%
  - Turn on all tanks of calibration gas to ~5-10psi
    - Excess flow should be apparent at T-fitting
    - **Paramagnetic O2 Sensor** – Turn on Nitrogen tank to ~5-10psi
  - If applicable, check connections on the column of white, granular Soda Lime
  - Click "CO2, CALIBRATE" to calibrate Carbon Dioxide Sensor
    - **Automatic CO2 Sensor** – If "Power" light is lit, but "Ready" light is not, call Columbus Instruments support

        - **Manual CO2 Sensor** – Adjust knobs to match displayed target values after purging; lock calibration knobs when finished
    - Click "O2, CALIBRATE" to calibrate Oxygen Sensor
        - **Fuel Cell O2 Sensor** – If the Coarse Gain knob needs to be turned more than half of a complete turn in between each calibration, the cell is near the end of its life; call Columbus Instruments to order a replacement
        - **Paramagnetic O2 Sensor** – Adjust knobs to match displayed target values after purging; lock calibration knobs when finished
        - **Zirconia O2 Sensor** – Adjust knob to achieve a ratio of 1.000; lock knob when finished
    - Turn off all calibration gas
- Configure experiment
    - Select an experiment configuration template; "File >> Open Experiment Configuration"
    - Fill out subject roster; "Experiment >> Setup"
        - Check the boxes for enabled rows
        - Enter in animal weights
        - Set fresh air flow; subjects sharing a flow controller will have identical flows
            - Mice: 0.50 LPM for ~20g subjects; 0.60 LPM for ~40g subjects
            - Rats: 2.0 LPM for ~200g subjects; 3.0 LPM for ~500g subjects
    - Choose a location on the hard disk to store experiment data; "Data Filename"
    - Choose a location on a network drive to backup experiment data; "Backup Directory"
    - **Telemetry** – Register E-Mitter transmitters to chambers; "Assign E-Mitters"
    - Click "Apply" when finished
    - Verify experiment settings; "Experiment >> Properties"
        - Choose reference method based on number of enabled cages (**5.1.1 Metabolic Measurements**)
        - Feed Control – Setup feeding schedule (**3.4.4 Feed Control Scheduling**)
- Run experiment; "Experiment >> Run"
    - Monitor first few readings to verify system is working properly
    - Verify reasonable In gas concentrations (~20.86-20.96% O2, ~0.040-0.080% CO2)
    - If possible, identify experiment room as "off limits"; limit traffic and other environmental variations
- Stop experiment; "Experiment >> Stop"
    - Fresh air will continue to be delivered to chambers while software is running
- Export data to CSV; "File >> Export >> Generate Subject CSVs"